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Diagnostics: Organs on a Vine

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WPI

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I. Introduction	LGG	LGG, JDJ, ALM, NRP, RET
II. Literature Review	LGG, ALM,NRP,RET	LGG, JDJ, ALM, NRP, RET
III. Project Strategy	LGG, NRP, RET	LGG, JDJ, ALM, NRP, RET
IV. Alternative Designs	LGG, ALM,NRP	LGG, JDJ, ALM, NRP, RET
V. Design Verification	LGG, JDJ, ALM, NRP	LGG, JDJ, ALM, NRP, RET
VI. Final Design & Validation	RET	LGG, JDJ, ALM, NRP, RET
VII. Discussion	JDJ, ALM	LGG, JDJ, ALM, NRP, RET
VIII. Conclusions & Recommendations	LGG	LGG, JDJ, ALM, NRP, RET
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Abstract

Drug screening is a complex and expensive process. Due to the limitations of preclinical testing, most drugs fail during clinical trials. No system currently available can determine a drug's effect on each organ in relation to one another. This project presents the novel use of several decellularized leaves seeded with human cells connected on the same recirculating perfusion system for drug screening purposes. Decellularization is a process in which cells are removed from the extracellular matrix (ECM). The ECM can then be used as a substrate for new cells to populate, which is a process known as cell seeding. In our system, each leaf is seeded with a different cell type. By interconnecting these leaves, we can study cell interactions for drug screening purposes. Testing has shown that our system can successfully decellularize spinach leaves, and human cells can be successfully seeded onto the surface of these leaves.

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I. Introduction

Pharmaceutical companies can spend vast amounts of money on the research and development of various chemical compounds [1]. Researchers must evaluate their compound's safety prior to clinical studies with *in vitro* studies assessing the impact on the major organ systems in the body. However, current methodologies fail to model the entire body's response. These systems also lack the microvasculature network that tissue engineering lacks, thus failing to accurately represent entire organs and organ systems for successful drug screening purposes.

The leaves that are present on the branches of plants have a very similar vasculature network to that of animals. By utilizing the natural similarities between plant and animal vasculature, it is thought that a plant based scaffold will provide a fully functioning vascular network that will supply the necessary oxygen and nutrients to the cells. Additionally, most leaves are connected to each other via a common vine or branch. By seeding different cell types onto different leaves on the same perfusion system, it is thought that the cells may be able to communicate with each other in a manner that is similar to the cell signaling process that occurs *in vivo*. This seeding of cells could lead to the development of organoids, which replicate organ functions and reactions to a drug or compound of choice. Creating a system where cells can communicate with each other similar to the process that occurs *in vivo* is of great interest to those involved in drug screening processes.

This novel idea of "organs-on-a-vine" incorporates different leaves that are present on the same vascular network. The different leaves were used as a scaffold for different animal cell types. The purpose of having the different cell types present on the same vascular network is to create a new drug screening tool. Current drug screening techniques are incapable of accurately modeling all organ systems as they are connected *in vivo*. A more accurate model can be created

by allowing different cell types to communicate through a connected perfusion system *in vitro*. This would allow for a more comprehensive drug screening process before drugs were to move into clinical trials.

By creating a new technology to mimic the *in vivo* drug response in an *in vitro* environment, the entire drug screening process would be revolutionized. The uncertainty that is involved before beginning clinical trials may be reduced when assessing systemic cell reactions in a system that models the entire human body. Additionally, this can reduce the chance of drugs or compounds failing in the late stages of clinical trials due to unforeseen reactions. The natural active vasculature that is present in plants may be very advantageous for drug screening purposes as there is an abundance of plants compared to the shortage and expense of fully functioning animal organs.

The overall goal of this project was to create a system that decellularize plants which also allowed the user to seed animal cells onto this newly created scaffold. This system was designed to mimic the drug response that would occur *in vivo* for animals in an *in vitro* environment. The different leaves were connected onto the same perfusion network so that the system would have circulating vasculature to encourage cell to cell communication. Additionally, the design had to maintain sterility, cell viability and structural integrity of the leaf throughout the entire process in order to be successful.

In order to achieve these goals, many different aspects and processes were involved. Initially, a system needed to be designed that would be able to perform both decellularization and allow for cell seeding. The design also had to connect plant material on the same vasculature network to encourage cell to cell communications. The design was drafted and discussed with

the client and all team members over multiple design iterations. Once a design was chosen, it was translated into a model that could be used for testing.

The first test that were conducted on the model of the chosen design was decellularization. Decellularization is the process of removing cells from an existing tissue in order to isolate the natural extracellular matrix (ECM). After the leaves went through the process of decellularization, human mesenchymal stem cells were seeded onto the different leaves. Complete cell media was perfused through the system in order to supply the cells with the necessary nutrients as well as create circulating vasculature.

The system is easy to use and satisfies the client's need. This project was initiated in order to create a system that could decellularize leaves and seed human cells onto them. Additionally, the cells and leaves needed to be connected on the same network to encourage cell to cell communication. It has been demonstrated that the system can successfully decellularize spinach leaves connected on the same vascular network. Once the leaves are decellularized, the user can seed human cells back onto the leaf in a sterile hood. Complete cell media can then be perfused through the leaves and into a collective reservoir, to then be perfused through the leaves again thus encouraging cell to cell communication.

By bringing this technology to the forefront of preclinical research techniques, areas of improvement were identified. Cellular reactions to compounds of interest could be quantitatively observed. Organoids could be created and connected on the same vascular network for drug screening purposes. This project has created opportunities in a new area of research involved the combination of plants and animals which may allow for the discovery of novel drug-screening technologies in the future.

II. Literature Review

2.1 Drug Screening

Pharmaceutical companies spend an average of 4 billion dollars on the research and development of a single approved drug [1]. Researchers must evaluate their compound's efficacy and more importantly, safety, prior to clinical studies. *In vitro* studies are completed to assess the impact of the drug on the major organ systems in the body. With approval from the Food and Drug Administration (FDA), the drug can proceed to clinical testing as can be seen in Figure 1 [2]. An accurate model for drug screening is necessary in order to prevent drug failure in the late stages of clinical trials.

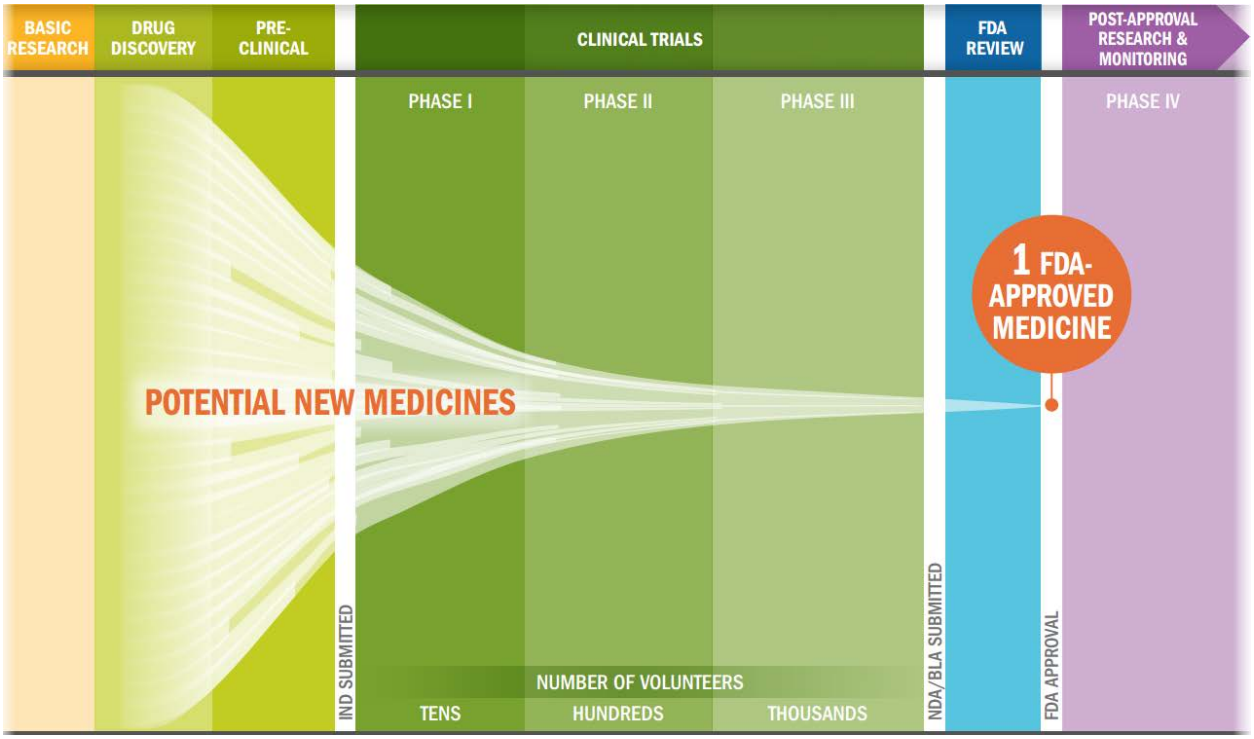


FIGURE 1. FDA APPROVAL PROCESS [27]

2.1.1 Current Technologies

Numerous *in vitro* drug screening assays assessing the potential effects of the compound must prove the drug's efficacy and safety prior to use in human subjects. At the most basic level,

two dimensional and three dimensional cell cultures are used for cytotoxicity and other assays.[3] Two dimensional cell culture is a very simple and widely used research method. Countless cells types can be seeded onto a flat surface under specific conditions where their responses can be assessed under a scope. As a method for drug screening, cells are placed on a plate and exposed to the compound of interest and examined for viability, proliferation rates and many other variables of interest. Three dimensional drug screening is a very similar experimental method only rather than seeding the cells onto the base of a plate, they are seeded into a three dimensional scaffold. These scaffolds can be made of collagen, hydrogels, and other unique materials. These methods, while being very cheap and very simplistic, are not a very good model for the complex human body. [4]

Another method for drug screening is high throughput screening (HTS), and is named for the ability to assay 20,000 or more compounds per week [4]. Companies that use this method must have a library of compounds to choose from and must optimize that library through theoretical medicinal chemistry prior to initiation of the process. The screening process involves multi-well plates (96, 384, or 1536) and robotic assistance. An assay, multiple biological targets, and numerous testing compounds must be chosen. This is often a rate-limiting step in the development of compounds for preclinical trials. The first pass screening qualitatively checks for reactions [4]. Only 0.1% to 1% of compounds express a positive result, and many of those can be false positives, therefore a second pass is run. This process includes verifying with other assays and dose response testing. Out of those that passed the first screen, only 1% will pass the second screen and many will be impractical for drug development, due to high toxicity or low potency. These impracticalities are addressed using theoretical medicinal chemistry to decrease toxicity

and increase potency of the compound. This is the limiting step in creating new compounds for preclinical testing [5].

In order to improve on the speed and functionality of HTS, research has been conducted into whole-animal screening. With the development of automated imaging, the worm *Caenorhabditis elegans*, the fly *Drosophila melanogaster*, and the fish *Danio rerio* can be used as whole animal models to screen compounds for drug creation [6]. With the use of animal models, the selection of particular biological targets can be eliminated, saving companies time and money. Additionally, progress has been made into the use of 3D human cell culture for HTS. As the 3D environment more closely mimics the *in vivo* environment, drug compounds chosen by this method are more effective in mouse studies [7]. However, this is an ethically controversial means of drug screening and there are several activist groups that oppose the use of animals for research purposes, one of which is People for the Ethical Treatment of Animals (PETA). For this reason many pharmaceutical companies are looking to different means of drug screening.

Organs-on-chips is the current gold standard in modeling the body for drug screening purposes. Organs-on-chips are multicellular microfluidic cell culturing chips that model the physiological functions of tissues and organs and the dynamic environment in which they rest [3]. Different cells types are cultured in their own continuously perfused micro-sized chambers from which channels extend outwards. Porous membranes separate each cell types' respective microchannels through which one can simulate and observe interactions at different tissue interfaces [3]. Cell chips have been developed over the past decade in order to study the effects that fluid flow and shear stress have on different cell types, including hepatocytes, cardiomyocytes, and cells from the kidney, skin, bone marrow, and lung [3].

The organs-on-chip design has the advantage of modeling cell to cell interactions *in vitro* so that the effects of pharmaceuticals can be tested and observed. The data that organs-on-chips produce help study the adsorption, distribution, metabolism, elimination, and toxicity (ADMET) of drugs on cells [3]. By determining these metrics, the effects of a drug on the system can be better understood. The organs-on-chips design allows further insight into the drug's pharmacokinetics and pharmodynamics. Pharmacokinetics is the study of the ADMET metrics over time in a system. Pharmodynamics looks into the relationship between drug concentrations and the positive and negative effects on the surrounding area [8]. Due to the high number of variables to study and observe, organs-on-chips are good diagnostic tools for pharmaceuticals to look at cell to cell interaction.

Organs-on-chips' microfluidic analog allows researchers to study how cells from different tissues absorb, distribute, metabolize, and excrete any chemical compound of interest thus increasing the accuracy of *in vitro* preclinical drug assessments [3]. The chip's customizability allows researchers to complete studies typically reserved for *in vivo* models. One can study, *in vitro,* systemic effects that a drug may induce by creating a biomimetic organism on a chip. Using this model for cell culture, the physical forces one might expect cells to be exposed to, such as mechanical compression, fluidic shear stress, and cyclic strain, can be tested and observed for different cell groups *in vitro.* Additionally, by simulating fluid flow and mechanical forces similar to those found *in vivo* one creates a more realistic *in vitro* model.

There are numerous limitations of organs-on-chips. They require specialized microengineering in order to fit the desired applications, for oftentimes one specific chip design is only suitable for one specific experiment. Additionally, the materials from which these chips are made often will absorb drugs being screened and render the desired test inadequate. The

required custom fabrication from potentially unique materials may drive the system price upward and decrease their availability for many researchers. They also lack the ability to express active vasculature which may be necessary for completing studies involving the circulatory and endocrine systems, as well as studies involving the interaction between cells and active small diameter blood vessels [3].

2.2 Modeling Active Vasculature

Understanding a drug's specific pharmacodynamics is vital during the completion of preclinical studies. Therefore, it is in an investigator's best interest to maximize the accuracy of the *in vitro* to *in vivo* extrapolation by modeling their study systems as closely to their ultimate *in vivo* counterpart.

In order to maximize an *in vitro* test's accuracy, especially during drug screening, the simulation of active vasculature is desired. A variety of active vasculatures found in mammals is a system of blood vessels with capillaries separated by approximately 220 μm [9]. Cells within the body are exposed to drugs via diffusion from a capillary through the extracellular matrix (ECM) as seen in Figure 2. Biologically driven attempts at modeling active vasculature for drug screening *in vitro* have been previously investigated. High rates of angiogenesis, or small diameter blood vessel growth, within zebra fish have encouraged scientists to use the entire organism as a drug screening model [10]. Their fast reproduction rate, ease of drug administration, and simplicity of studying systemic effects make this a desirable substitute model for rats and mice. By repurposing existing tissues, such as that from plants, an *in vitro* system for drug screening which exhibits active vasculature can be developed.

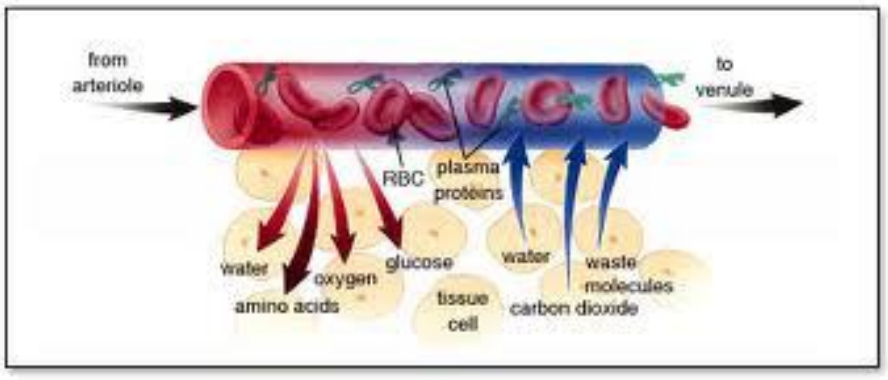


FIGURE 2. DESCRIPTION OF DIFFUSION AND TRANSPORT PROCESSES IN VASCULARIZED TISSUES [28]

2.3 Plant and Animal Cell Structure

One of the largest differences between plant and animal cells is the presence of the cell wall. The main differences between plant and animal cells are described in Table 1.

TABLE 1. DIFFERENCE BETWEEN PLANT AND ANIMAL CELLS

Plant Cells	Animal Cells
Presence of a cell wall	Cell membrane only
2 vasculature networks do not connect	Recirculating vasculature
ECM is mainly composed of cellulose	ECM is mainly composed of collagens
Vasculature is constructed out of the interior of non-functional cells	Vasculature is a tube constructed from cells, often with supporting musculature

The cell wall gives plant cells strength and support and prevents them from bursting from osmotic pressure. The cell wall is composed of mainly polysaccharides in two layers. The primary outer layer is composed mostly of cellulose, hemicelluloses, and pectins. The

hemicelluloses, pectins, and a variety of glycoproteins bind the cellulose fibers together, giving the cell wall strength. This layer regulates intercellular transport and is flexible enough to allow growth. The secondary layer is thicker and stronger, composed of 50 to 80% cellulose as well as lignin, which is a very strong polymer. This layer supports the cell more than the primary layer [11]. Connecting all the cells together is the middle lamella, primarily composed of pectins [12]. The cell wall and middle lamella are comparable to animal cell's extracellular matrix. A diagram of plant cell anatomy can be found in Figure 3.

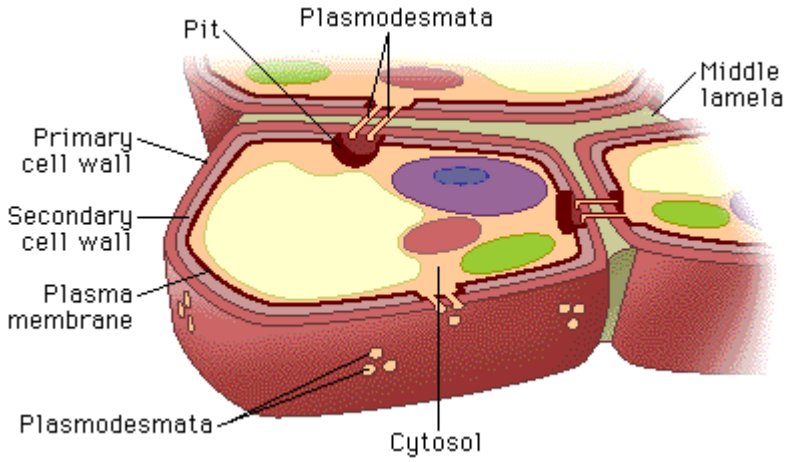


FIGURE 3. PLANT CELL ANATOMY [30]

Animal ECM is primarily composed of collagen, which is made of amino acids. There are several different types of collagen, as the composition varies. ECM also contains elastin, which allows it to stretch. Animal ECM also includes polysaccharides, though to a lesser extent than plants. There are glycosaminoglycans, which are similar to pectins in that they trap water to form gels. These are linked to a protein in order to form proteoglycans, which function in cell to cell signaling and cell-matrix adhesion [11].

Plant tissues include leaves, stems, and vascular networks. The vascular networks are the xylem, which carries water and dissolved minerals from the roots to the leaves, and the phloem,

which carries food made by the leaves to the rest of the plant. These two networks do not connect, unlike animal vasculature, in which the vascular network forms a recirculating system [13]. A histological stain of plant vasculature can be seen in Figure 4.

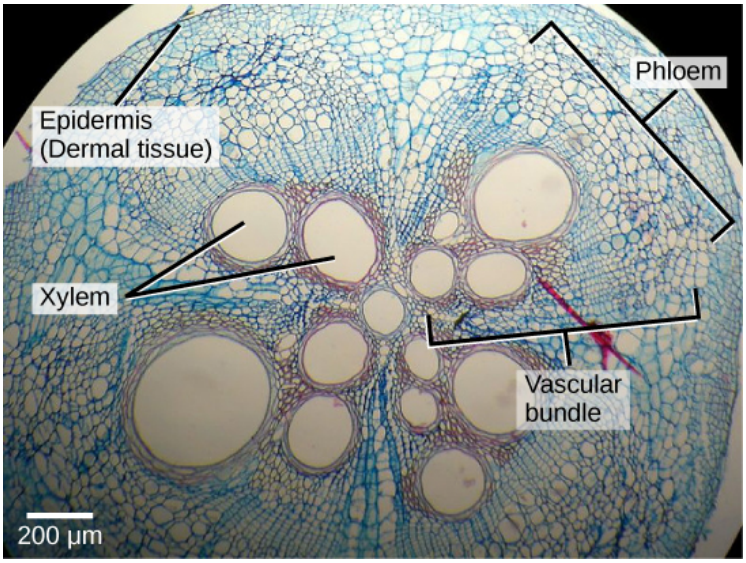


FIGURE 4. HISTOLOGICAL IMAGE OF PLANT VASCULATURE [21]

Xylem is composed of vessels, tracheids, fibers and parenchyma cells [14]. A vessel is a length of several dead cells that are connected so that water may flow through them. A single vessel is perforated at either end to connect to the next. Tracheids are also dead cells that conduct water, though they are individual cells and move water at a slower rate as each cell is not perforated or connected to the next. Vessels are the primary method of water transportation in angiosperms, flowering plants whose seeds are located in fruit [15]. Fibers and parenchyma cells are still alive and act as structural support.

Phloem is composed of sieve tubes, companion cells, fibers, and parenchyma cells [16]. Sieve tubes are a series of cells connected by perforated cell walls. These cells lose their nuclei and other organelles but retain their cytoplasm, plasma membrane, mitochondria, and endoplasmic reticulum. These tubes are always accompanied by companion cells, specialized

parenchyma cells that keep the sieve tubes alive [16]. Like xylem, the fibers and parenchyma cells give the phloem structural support.

The phloem and xylem are separated by the cambium, which are fully functional cells. The cambium is responsible for the radial growth of the plant. A representative image of a plant's vasculature, including the xylem, cambium and phloem can be seen in Figure 5.

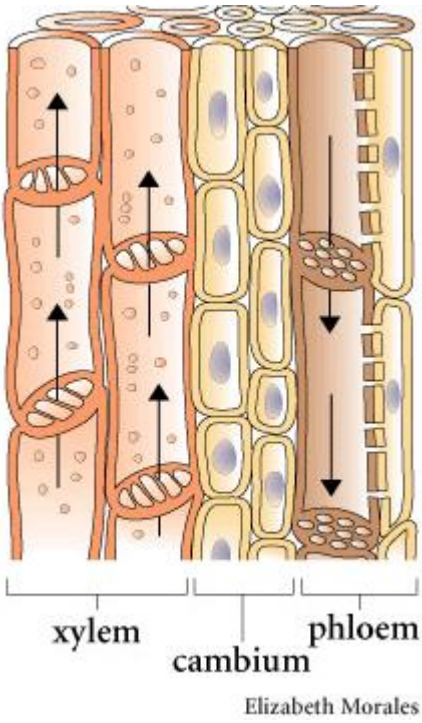


FIGURE 5. PLANT VASCULATURE [21]

Wastes are released in gaseous form through stomata on the underside of the leaves. In flowering plants, the xylem and phloem are organized as monocots or dicots. Monocots have bundles of xylem and phloem scattered around the center of the stem. In dicots, the xylem and phloem form concentric circles [13].

The xylem and phloem are continuous from the central stem into a plant's leaves. An individual leaf stem is called a petiole, which is often used for decellularization as it has a wide opening. From the petiole, the vasculature of the leaf has two forms of branching: netted or

parallel. Like the names suggest, netted vasculature is seen to continually branch across the surface of the leaf, as seen in spinach, while parallel vasculature forms parallel lines along the length of the leaf, as seen in a blade of grass [29].

Conversely, animal vasculature is composed of a layer of endothelial cells and at higher levels, smooth muscle cells. As the system is regularly subjected to fluctuating pressures, the muscle cells lend the ability to withstand those pressures while still allowing flexibility. Plant vasculature only flows in one direction, whereas animal vasculature recollects metabolic wastes and filters them separately.

Overall, plants have a number of classification systems. A useful one is by growth form, divided into trees, shrubs, and herbs. Herbs are the least woody, meaning the cells have the thinnest cell walls and the least mechanical strength [17]. These types of plants are preferable for decellularization, as fluids do not require as much time to perfuse through a leaf as for trees or shrubs.

2.4 Decellularization

Decellularization refers to the process in which cells are removed from a tissue or organ in order to obtain the ECM for use as a scaffold. This process is widely studied and implemented for tissue engineering and regenerative medicine applications. Once the ECM is obtained as a biological scaffold, it can be used to host new cells for various applications such as implantation, research purposes, and potentially, drug screening [18]. There are clinical products on the market such as surgical mesh materials that are composed of ECM and harvested from a variety of allogeneic (same species) or xenogeneic (between species) tissue sources. It has been proved that the ECM influences cell mitogenesis and chemotaxis, directs cell differentiation, and induces

constructive host tissue remodeling responses. Therefore, decellularization of different tissues is essential in order to create clinical products to be used in the body [19].

The main goal of decellularization is to remove all cellular and nuclear material from the tissue or organ of interest, while minimizing any adverse effects to the remaining ECM. Due to the rigorous nature of the decellularization process, which involves both physical and chemical disruption of the tissue or organ of interest, the composition, biological activity or mechanical integrity of the ECM may be disrupted. Most decellularization processes attempt to minimize the disruption to the ECM in order to retain its' natural properties while also ensuring that the tissue or organ can be completely rid of the cellular and nuclear components [18].

It is important that the tissue or organ is decellularized, opposed to directly implanting a tissue to another patient in need, due to the inflammatory or immune-mediated rejection of the tissue. Most xenogeneic and allogeneic cellular antigens are recognized by the host body as foreign and will elicit the inflammatory or immune-mediated rejection. In order to prevent this response from happening, decellularization is used to remove the foreign cells from the scaffold of interest. The ECM and its' components however, are generally conserved among species and will not elicit the inflammatory or immune-mediated rejection [18].

The decellularization process typically includes a combination of physical, chemical and enzymatic methods. The process generally begins by lysing the cell membrane, followed by separating the cellular components from the ECM using enzymatic treatments, solubilizing the cytoplasmic and nuclear components using a detergent and finally removing all cellular debris from the tissue [18]. All of the previously listed steps can be combined with mechanical agitation to gain more efficient and effective results. Any residual chemicals must be removed from the tissue or organ that is being decellularized in order to reduce any response from the host tissue

where it will eventually be used. This is usually done by flushing the tissue or organ with distilled water. Table 2, from *Decellularization of Tissues and Organs* [18], summarizes the commonly used decellularization methods and chaotropic agents. It is important to note that the exact decellularization process that is used will be dependent on the tissue or organ of interest.

TABLE 2. COMMONLY USED DECELLULARIZATION METHODS AND CHAOTROPIC AGENTS FROM DECELLULARIZATION OF TISSUES AND ORGANS [12]

Method	Mode of Action	Effects on ECM
Physical		
Snap freezing	Intracellular ice crystals disrupt cell membrane	ECM can be disrupted or fractured during rapid freezing
Mechanical force	Pressure can burst cells and tissue removal eliminates cells	Mechanical force can cause damage to ECM
Mechanical agitation	Can cause cell lysis, but more commonly used to facilitate chemical exposure and cellular material removal	Aggressive agitation or sonication can disrupt ECM as the cellular material is removed
Chemical		
Alkaline; acid	Solubilizes cytoplasmic components of cells; disrupts nucleic acids	Removes GAGs
Non-ionic detergents		
Triton X-100	Disrupts lipid–lipid and lipid–protein interactions, while leaving protein–protein interactions intact	Mixed results; efficiency dependent on tissue, removes GAGs
Ionic detergents		
Sodium dodecyl sulfate (SDS)	Solubilize cytoplasmic and nuclear cellular membranes; tend to denature proteins	Removes nuclear remnants and cytoplasmic proteins; tends to disrupt native tissue structure, remove GAGs and damage collagen
Sodium deoxycholate		More disruptive to tissue structure than SDS
Triton X-200		Yielded efficient cell removal when used with zwitterionic detergents
Zwitterionic detergents		

CHAPS	Exhibit properties of non-ionic and ionic detergents	Efficient cell removal with ECM disruption similar to that of Triton X-100
Sulfobetaine-10 and -16 (SB-10, SB-16		Yielded cell removal and mild ECM disruption with Triton X-200
Tri(*n*-butyl)phosphate	Organic solvent that disrupts protein–protein interactions	Variable cell removal; loss of collagen content, although effect on mechanical properties was minimal
Hypotonic and hypertonic solutions	Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively remove the cellular remnants
EDTA, EGTA	Chelating agents that bind divalent metallic ions, thereby disrupting cell adhesion to ECM	No isolated exposure, typically used with enzymatic methods (e.g., trypsin)
Enzymatic		
Trypsin	Cleaves peptide bonds on the C-side of Arg and Lys	Prolonged exposure can disrupt ECM structure, removes laminin, fibronectin, elastin, and GAGs
Endonucleases	Catalyze the hydrolysis of the interior bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Exonucleases	Catalyze the hydrolysis of the terminal bonds of ribonucleotide and deoxyribonucleotide chains	

One example of the decellularization process is the decellularization of hearts using methods similar to that described by H.C. Ott [14]. The aorta feeds into the coronary arteries which supply the cardiac muscle with blood. This makes the aorta an ideal location to perfuse the decellularization agents. First, the aorta of the heart is isolated, cannulated, and connected to a bioreactor. The heart is then perfused with heparinized phosphate buffered saline (hPBS) for 1 hour followed by 1% sodium dodecyl sulfate (SDS) in deionized water for 48 hours. The heparinized PBS is used to breakup blood clots and thin any blood left in the heart. The SDS serves as the primary decellularizing agent. After SDS, The heart is perfused deionized water (DI

water) for 12 hours. Following the deionized water 1% triton-X in deionized water is perfused for another 12 hours. To help prevent contamination, the heart is perfused with phosphate buffered saline treated with Penicillin, streptomycin, and amphotericin (aPBS) for 48 hours. Decellularization is then visually confirmed by a translucent appearance of the cardiac ECM. This entire heart decellularization processes is summarized in Figure 6[14].

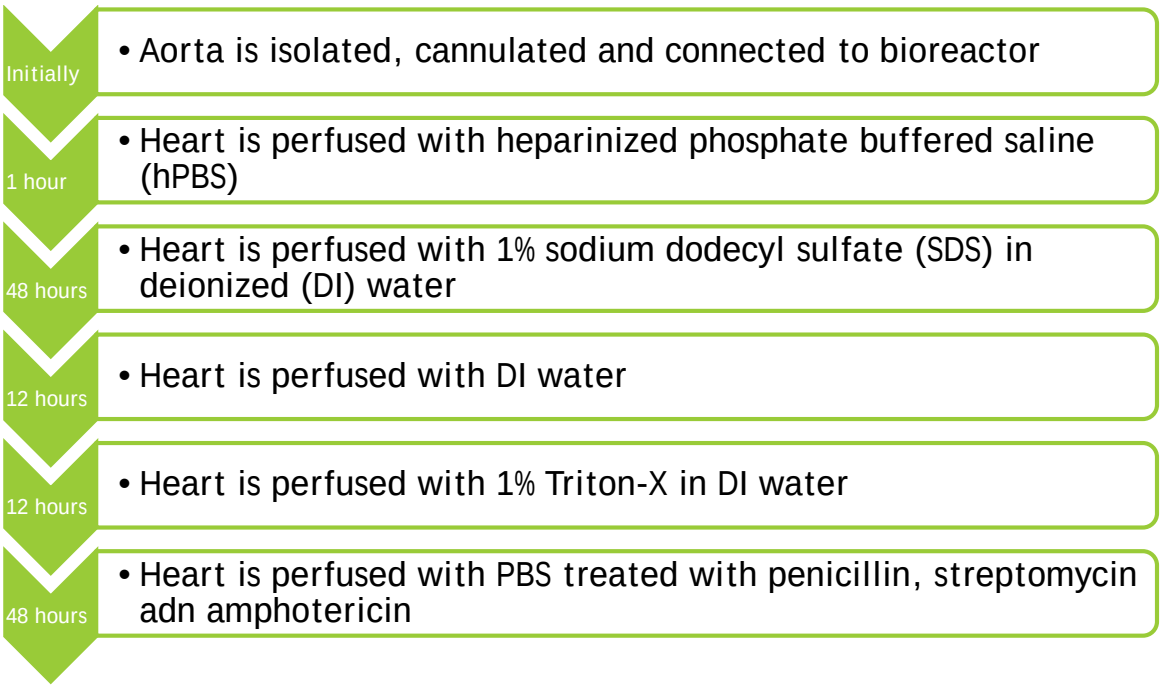


FIGURE 6. HEART DECELLULARIZATION PROCESS

This process can also be visualized in Figure 7, in which the entire decellularization process is complete in 60 hours.

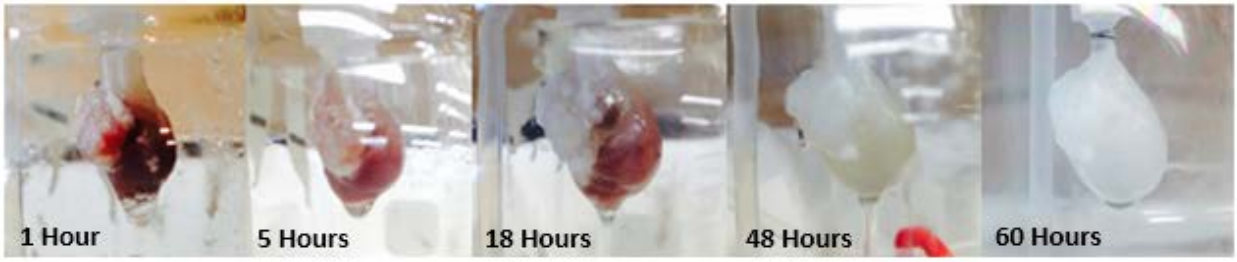


FIGURE 7. TIME LAPSE OF HEART DECELLULARIZATION

In Figure 7, the decellularized heart appears clear after 60 hours which indicates that the decellularization process is complete. However, it still must be confirmed that there is an absence of cells. This is done through fluorescence imaging and staining, which can be seen in Figure 8.

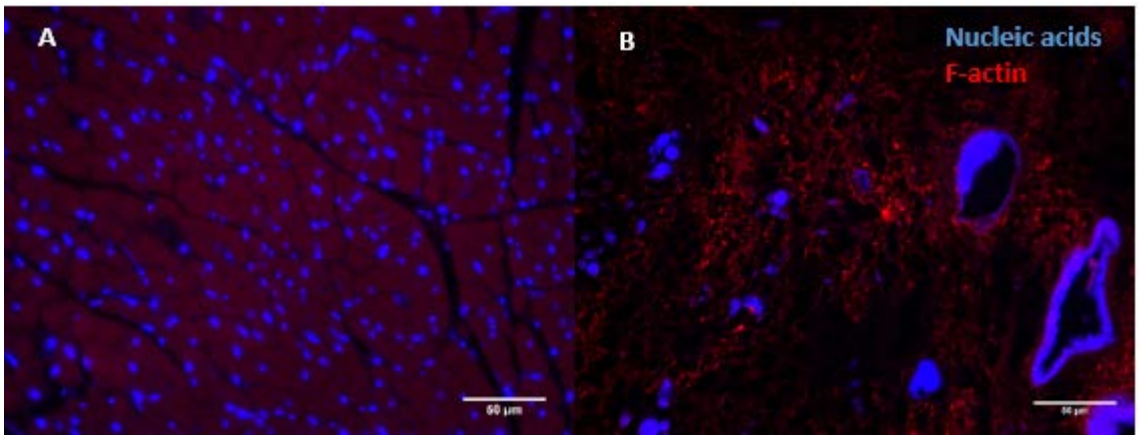


FIGURE 8. (A) 40X IMAGE OF NORMAL MYOCARDIUM STAINED WITH HOECHST (BLUE) PHALLOIDIN (RED) (B) 40X IMAGE OF DECELLULARIZED MYOCARDIUM STAINED WITH HOECHST (BLUE) AND PHALLOIDIN (RED)

Figure 8 represents the microscopic images of a normal myocardium and a decellularized myocardium stained with Hoechst and phalloidin at 40x magnification. Hoechst is a stain that will stain nucleic acids blue, thus staining the nuclei blue. Phalloidin is a stain that will stain F-actin red, thus staining the cytoskeleton and contractile cells red. As the image on the left of the normal myocardium has many blue dots present, it is clear that there are many cells present. As the image on the right does not have any defined nuclei, it is clear that there are no cells present. The blue stain that can be seen in Figure 8B is nuclear material left behind in the arterioles and venules from incomplete washing of the heart. This image confirms a successful decellularization process in which the cells are removed from the extracellular matrix.

2.5 Plant Decellularization

As previously stated, plant decellularization is slightly different than the decellularization of hearts. The most successful plant decellularization has been performed on spinach leaves due

to their large petioles. The main differences between heart and spinach leaf decellularization primarily affect the perfusion time of the decellularization agents. Spinach leaves have a cuticle, which is a naturally waxy coating on their surface. Before the leaf can be decellularized, the cuticle must be removed. To remove this coating the leaf must first be submerged in hexanes and rinsed vigorously with PBS. This is done three times to ensure that the cuticle has been completely removed. The stem of the leaf is then cannulated and secured to the needle with glue or a suture. Once the leaf has been properly prepared it is connected to the setup and perfused with 10% SDS for 5 – 7 days. The leaf is then perfused with a solution of 0.1% Triton-X 100 and 10% bleach for 24 – 48 hours. At this point decellularization is confirmed by a translucent appearance of the leaf. Deionized water is then perfused through the leaf for 24 hours. This entire process is summarized in Figure 9.

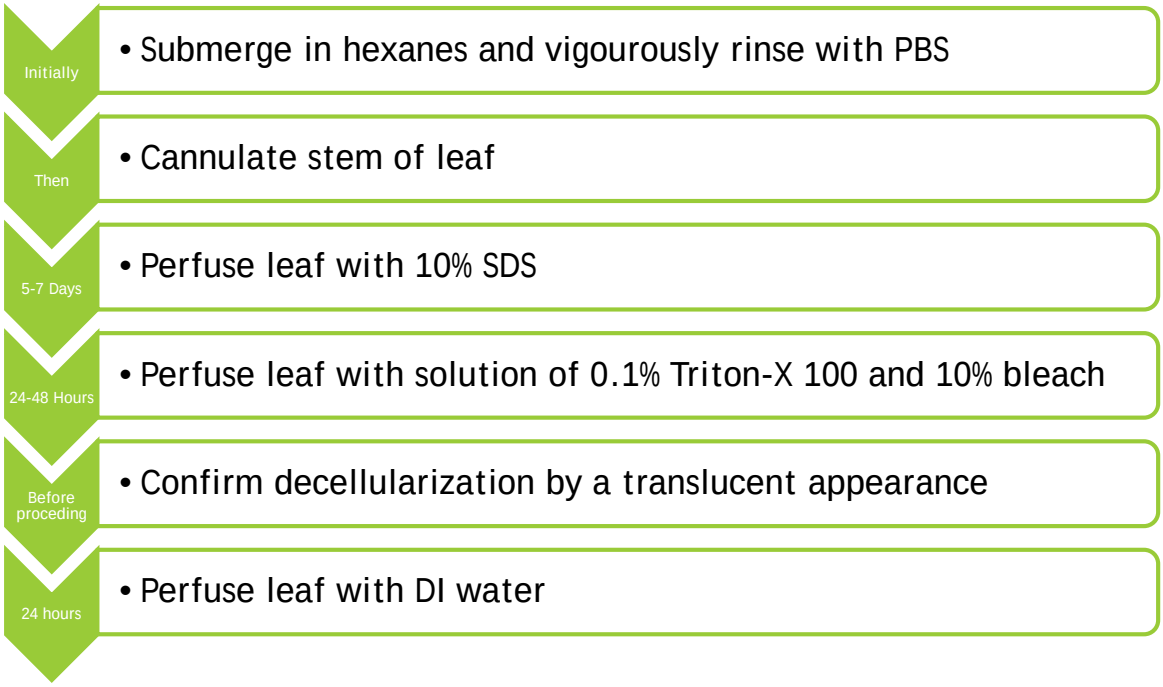


FIGURE 9. PLANT DECELLULARIZATION PROCESS

2.6 Cell Seeding

Cell seeding refers to the process in which cells are placed onto an external environment on which they will grow. Cell seeding is usually done by placing a predetermined number of cells onto a specific area of space to allow for cell growth. In order to seed cells onto a leaf, a defined protocol must be followed. First it must be ensured that the leaf is decellularized and sterilized. The leaf is sterilized during the last step of decellularization in which the leaf is perfused with 0.1% Triton-X 100 and 10% bleach for at least 24 hours until the leaf becomes translucent. Next, the leaf is perfused with sterile deionized water to rinse all of the decellularization agents and bleach from the leaf and the perfusion system for 24 hours. Once the perfusion system is cleared, all perfusate is aspirated and replaced with complete cell media. The media is perfused through the perfusion system and the leaf for 24 hours while in the incubator in order to equilibrate the leaf to the salinity of the media. After incubation, the leaf was chelated with 2mM EDTA in PBS. Next, the leaf is secured to the tray using a sterile washer. This ensures that the leaf surface is taught and solid to the tray. Finally, 150,000 cells are seeded onto the surface of the leaf within the washer. Once the cells are seeded, the entire perfusion system is placed back into the incubator to allow the cells to grow. This entire process is summarized in Figure 10.

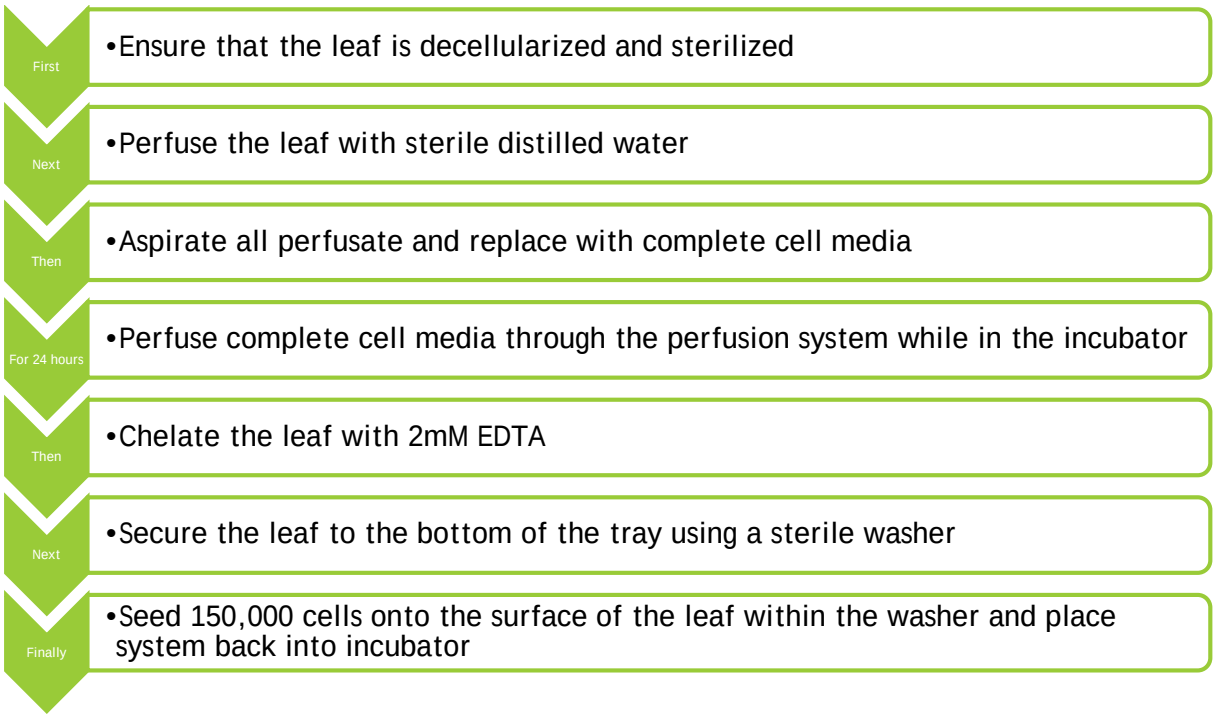


FIGURE 10. PROCESS FOR SEEDING CELLS ONTO A LEAF

III. Project Strategy

3.1 Initial Client Statement

The client for our design is the Gaudette Lab. More specifically, our direct clients with which we had regular contact with were Professor Gaudette and a graduate student from the Gaudette Lab, Joshua Gershlak. The Gaudette Lab has already been able to decellularize certain leaves (such as spinach leaves) and use them as scaffolds for animal cell culture. However it has not been determined if these cells that were seeded were viable. By completing this first step in the process, our client, the Gaudette Lab sought to apply this technology for drug screening purposes. In order to achieve this goal, Professor Gaudette provided the team with this initial client statement:

> **"One such approach that we find that could be exciting would be a systems biology approach or "organs on a vine". By decellularizing multiple leaves**

on the same vascular network or vine/branch, we could successfully design fully biomimetic body on a branch. Different cell types could be seeded on different leaves or portions of the network in a way that mimics physiological systems *in vivo*. Potential applications of this designed "organs on a vine" would be for drug screening and physiological testing."

This provided client statement allowed the team to begin thinking about the possible features of the designed product.

3.2 Design Requirements: Technical Objectives

Design objectives were created based on preliminary research and the client statement. Objectives are defined as "a feature or behavior that the design should have or exhibit" [20]. The list of objectives can be seen below listed in no particular order.

- Protect structural integrity of the leaf throughout the entire process
- Able to facilitate plant decellularization and cell seeding
- Host multiple cell types on multiple leaves
- Model active vasculature using plant anatomy
- Provide stable extracellular substrate

1. Protect structural integrity of the leaf throughout entire process

The design must protect the structural integrity of the leaf throughout the entire process. If the leaf becomes compromised at any point during use with the design, perfusion will not occur properly which may result in incomplete decellularization or cell death. The leaves become extremely fragile once decellularization occurs and this must be taken into account when designing a system to hold the leaves.

2. Facilitate plant decellularization and cell seeding

The design must facilitate decellularization and cell seeding of plants. This is a very important objective of the system as it will allow the system to act as a "two-in-one device". Decellularized plants have natural vasculature that is very similar to that of an animal and the client wants to take advantage of this feature. It is believed that the vasculature of the plant itself will allow for cell communication similar to that which occurs in vivo in animals. By both decellularizing the leaf and seeding cells onto the leaf with one device, the entire process can occur in one place. This will also reduce the risk of sterility compromises or breakage of the leaf as it will remain within one device.

3. Host multiple cell types on multiple leaves

The system must incorporate different cell types that are physically isolated from one another. Different cell types are needed so that there can be communication between different cell types that produce an observable reaction. The cellular communication can then be used as in *in vitro* model for drug screening as stated in the client statement.

4. Model active vasculature using plant anatomy

The design must model active vasculature using plant anatomy. Active vasculature is the main reason why other drug screening models have failed so it is important to incorporate them into our design. The active vasculature present within our design will also more closely mimic *in vivo* conditions than other drug screening models.

5. Provide stable extracellular substrate

The design must allow for a stable extracellular substrate. In this case, the extracellular substrate is the leaf itself so the design must ensure that the leaf is stable and protected. If the leaf is in jeopardy of being compromised in any way, such as tearing, the cells may also be in jeopardy which may negatively affect our design validation and any subsequent testing.

Constraints

Preliminary research, along with the client statement, were used to define design constraints. A constraint is defined as "a limit or restriction on the features or behaviors of the design. A proposed design is unacceptable if these limits are violated" [20]. Below are a list of the design constraints listed in no particular order.

- Maintain cell viability
- Must facilitate decellularization and cell seeding (maintain structural integrity of plant)
- Must be sterilizable and maintain sterile environment
- Provide/support a non-toxic extra-cellular matrix (ECM)
- Must fit in incubator
- Must be within budget

1. Maintain cell viability

The first design constraint is to maintain cell viability. This is essential as the cells must be able to live and survive on the system that is created. If the cells do not survive, the system cannot be utilized for its intended purposes of observing the interactions between different cell types.

2. Must facilitate decellularization and cell seeding while maintaining structural integrity of the plant

The second design constraint is that the system must facilitate decellularization and cell seeding while maintaining the structural integrity of the plant. While these were two separate design objectives, together they form one design constraint. If the system cannot facilitate decellularization and cell seeding while maintaining structural integrity of the plant, then the

system will have failed. The main motivations from the client were to create a device that would successfully recreate circulating vasculature to mimic *in vivo* conditions in an *in vitro* environment. If the plants are compromised or cells cannot be seeded onto them, then this unique environment cannot be created and the system will have failed.

3. Must be sterilizable and maintain sterile environment

The third constraint is that the system must be sterilizable and maintain a sterile environment. This means that the system must be able to be sterilized through established means of sterilization without compromising the system. If the system cannot be sterilized through previously established sterilizing methods, then the system cannot be used for cell culture. Additionally, the system must maintain the sterile environment once it has been established. This is important as cell culture requires a sterile environment in order for the cells to survive without contamination.

4. Provide/support a nontoxic extra-cellular matrix (ECM)

The fourth constraint is that the system must provide/support a nontoxic extracellular matrix. This is important as the cells need to be able to survive on the system. If the plant were to provide a toxic ECM, the cells may not be able to survive. Additionally, if the ECM were toxic due to decellularization agents that were not properly flushed from the system, the system would likely kill the cells and the system would fail.

5. Must fit into incubator

The fifth constraint is that the system must fit/live in the incubator. This is a major constraint of the system as the incubators that are provided in Gateway Park and Goddard Hall at WPI are a specific size and the incubation space must be shared with other researchers in the lab. The system will have to fit inside the incubator provided so that the cells may maintain vitality.

6. Must be within budget

The sixth and final constraint is that the system must be within budget. A set budget of 625 USD has been established for this project that cannot be exceeded in order to create the final design. This budget is derived from the Biomedical Engineering Department at WPI, which allots 125 USD per student. Additionally, if this system were to be implemented by other users, it needs to be cheaply manufacturable so that they can recreate our system without cost being a major issue.

Functions

Preliminary research, along with the client statement, were used to create and define design functions. Functions are defined as "those things a designed device or system is supposed to do" [20]. The list of functions can be seen below listed in no particular order.

- Decellularizes the plant
- Allows user to seed cells on the plant
- Recirculates media
- Maintain flow to multiple leaves
- Allows for variable flow rates between samples
- Transportable
- Allows user to introduce compounds to the system

1. Decellularizes the plant

As previously mentioned in both the objectives and constraints, the design must be able to decellularize the plant material. This is a major function and is essential to the success of the design and its functionality. If the design cannot successfully decellularize the plant, then it is failing to meet the client's needs and expectations.

2. Allows user to seed cells on the plant

Our design must also allow the user to seed cells onto the plant. Similar to decellularization, this is a major component and is essential to its success and functionality. Unlike decellularization, our design does not need to physically seed the cells on the plant itself, rather allow the user to seed cells on the plant.

3. Recirculates media

Our design must recirculate media throughout the entire system. In order for our design to model active vasculature and attempt to mimic *in vivo* conditions, recirculation of media is essential to transport cell signals and nutrients to all of the cells on different leaves. The recirculating media acts as a model of blood *in vivo*, which serves to transport cell signals and nutrients in the body.

4. Maintain flow to multiple leaves

Our design must be able to maintain flow to multiple leaves. This is important because it is desired that multiple leaves are used to separate different cell types. Our design must be able to provide media to the different leaves in order to keep the cells alive. However, it is important that the flow is not specific to one leaf, but rather, divided among all the leaves in order to encourage cellular interactions.

5. Allows for variable flow rates between samples

Our design should also allow for variable flow rates between samples. In the human body when blood is pumped from the heart, each organ receives a different percentage of the blood. In order to model *in vivo* conditions, the flow rate and overall percentage of the media that goes to the different leaves should be controllable. This is because the different leaves may have different cell types from various human organs thus creating a more accurate model.

6. Transportable

The design must be transportable without compromising sterility. This is important because the system must be able to move from the sterile hood to the incubator once seeded with cells in order maintain cell viability. This also means that the design must be relatively compact so that the team can easily move the system from one location to the other.

7. Allows user to introduce compounds to the system

Finally, the design must allow the user to introduce compounds to the system. This will ultimately facilitate the drug screening process. Cellular reactions from these compounds can be observed in order to determine the compounds/drugs effect on those specific cells and the human body overall.

3.3 Design Requirements: Standards

The ISO (the International Organization for Standardization) is responsible for ensuring that the testing of medical devices is completed in respect to an internationally agreed upon standard. It is important that our device and associated testing and maintenance procedures satisfy each ISO requirement. Section 3.7 of ISO 13485:2003 characterizes our plant based perfusion system as a medical device in that it "provides information for medical purposes by means of an in vitro examination of specimens derived from the human body". The cells to be used as a diagnostic substitute in our device are specimens derived from the human body, therefore our entire system must satisfy all relevant ISO requirements of a medical device [22].

Sterilization

To ensure that a medical device is free of any and all viable microorganisms one must expose it to certain conditions from a validated sterilization method and it must be observed and reviewed for sterility. ISO 11137 and ISO 11135 outline numerous means of validating the sterility of our device. The most relevant means are the Direct Transfer Sterility Test, in which

the sterile portion of the device is submerged in a test media which is then observed for microorganisms. Additionally, the Product Flush Sterility Test, in which a contact media is perfused through any small diameter tubing and then observed for microorganisms, must be completed. This contact media shall then be sent to a qualified third party for interpretation [23].

Drafting

In developing a medical device, one must satisfy ISO 129-4:2013. This outlines a series of guidelines for technical drawings regarding their indications of dimensions, tolerances, and formation of draft sheets. This is done to maximize the conveying of design intention. This standard outlines the rules of presentation for the numerous dimensioning scenarios. This also describes the proper positioning of dimensions, the staggering of dimensions, the units to be used and general tolerances. It also describes the means of indication of special dimensions such as grooves, chamfers and many more. SolidWorks, the software used for the drafting of our system, allows default dimensioning and drafting options of ISO [24].

Electronic controls

Our system is run by a pumping system that is controlled by an Arduino microcontroller. These controllers are an open source system that has been granted a Federal Communications Commission (FCC) equipment certification for complying with any requirements that are applicable to its scope of function [25]. Arduino coding was done using their own Integrated Development Environment (IDE). Coding methodologies and techniques were completed in compliance with the Indian Hill C Style and Coding Standards paper [26].

3.4 Revised Client Statement

Considering the most important design objectives, constraints and functions, the project goals were further established and the following revised client statement was created:

“Design a system for the decellularization and cell seeding of plants to model tissues in the body and their active vasculature for drug screening purposes. It would be ideal to acquire data on cellular reactions to compounds of interest. The design must maintain sterility, cell viability and structural integrity of the leaf throughout the entire process. ”

This revised client state clearly defines what the system must do and will help to shape the final outcomes of the design in order to satisfy the client.

3.5 Management Approach

Technical Approach

After the revised client statement was identified, objectives, functions and constraints were defined so that a project approach could be formulated. Initially, the team went to the Gaudette Laboratory to see the plant decellularization process that is currently implemented. It was intended that this approach would also be used for the completion of this project. Preliminary research was also conducted on different plants, the decellularization process and current *in vitro* models for drug screening. More specifically, the organs-on-chips technology was researched and its limitations were noted.

After preliminary research was conducted, the revised client statement was established and the objectives, constraints and functions were identified, the creation of alternative designs began. These alternative designs aimed to satisfy the revised client statement, while also satisfying the objectives and functions of the design and adhering to the identified constraints. These alternative designs were proposed by different members of the team which were recorded and subsequently presented to the client for further feedback.

After discussing the pros and cons of each design with the client, a final design was selected for prototyping. The mechanical part of the design was first modeled using SolidWorks, which was then manufactured from acrylic sheets using a laser cutter. The different pieces of acrylic were mated using acrylic cement. The electrical system of the design is controlled by an Arduino microcontroller. By combining the electrically controlled pump and fans with a fluid recirculation system, the prototype was created. This prototype was then used for design testing and validation.

Management Approach

In order to maintain good communication between the design team and the clients (Professor Gaudette and Joshua Gershlak), a weekly meeting schedule was established. This was vital in updating the clients on the progress of the project and reviewing the work that was previously completed. This meeting was also used as a time for discussion in which ideas and questions could be communicated. In order to keep the project on track, milestones were created in terms of the project management using a Gantt chart, which can be seen in Appendix E: Gantt Chart. There are many major milestones included: defining the client statement, defining objectives, constraints, functions and means, generating design alternatives, choosing a design, design construction, design validation and data collection, and finally data analysis.

IV. Design Process

4.1 Needs Analysis

Chapter 3 outlines the objectives that were established from the revised client statement. In order to prioritize these objectives for the design process a weighted objectives scale was created, in which each objective was given a score from 1-10, 1 indicating low priority and 10 indicating necessity for the function of the device. The objectives with their respective scores can be seen in Table 3.

TABLE 3. WEIGHTED OBJECTIVES SCALE FOR THE FINAL DESIGN

Objectives	Score
Able to facilitate plant decellularization and cell seeding	10
Protect structural integrity of leaf	9
Ability to host multiple cell types on multiple leaves	8
Model active vasculature using plant anatomy	10
Provide stable extracellular substrate	8

The first objective “able to facilitate plant decellularization and cell seeding” was given a score of 10 as it is necessary for function of the device. Decellularization and cell seeding are the two main functions of the device and if the design does not allow for these functions to occur, then the device will have failed. The design must also “protect the structural integrity of the leaf”, but was given a 9 on this weighted objectives scale. It is important that the structural integrity of the leaf is protected so that the cells seeded on the leaf survive. However, the entire leaf does not

need to be protected, only the stem and the area with the seeded cells which is why this objective received a score of 9 instead of a 10.

The design must also be able to “host multiple cell types on multiple leaves”. This objective was given a score of 8 because this will allow for the creation of organoids and subsequent drug screening. However, this project was centered on testing one leaf, allowing for the use of multiple leaves in the future. The next objective is to “model active vasculature using plant anatomy” which was given a score of 10. This is essential to the design as the utilization of plants was the client’s initial motivation for this project. Additionally, plant material has active vasculature that current drug screening technologies have limited capacity in recreating.

The last objective is to “provide a stable extracellular substrate”. This objective was given a score of 8 because the design may be able to succeed if the extracellular substrate was not stable, however it would make the design very difficult to handle. For example if the leaf were not supported, the cells may still survive, but there is a possibility the structural integrity leaf may be damaged due to transportation. Using the weighted objectives scale in Table 3, conceptual designs were drafted in order to satisfy the objectives for this design.

4.2 Conceptual Designs and Feasibility Studies

The team established various conceptual designs that would allow for both the decellularization and cell seeding of plant material with recirculating vasculature. It was essential that these conceptual designs would have the capacity to adhere to all of our defined objectives, constraints, and functions. Descriptions of these conceptual designs are provided in the following sections along with each designs advantages and limitations. Feasibility studies were also

conducted in order to determine if specific aspects of our design would be feasible. The set-up and results of these studies are described in the following sections.

Conceptual Design 1: Utilization of Leaves on a Vine

One of the team's conceptual designs involved using an entire vine, with the leaves included, as the plant material that would be used in this system. This design would be connect the vine with itself using a pump and sterile tubing in order to create a recirculating system. A single collection bin would sit under the vine and collect the perfusate which would feed back into the pump and throughout the entire system.

One advantage of this system is that it utilizes the entire vine. All of the available plant anatomy would be used and would satisfy the client's initial motivations. The disadvantages to this system are that the actual vine only provides a one way directional flow due to a plant's natural anatomy. Additionally, if a vine were to be used, it would be fairly difficult to control the rate of flow to the different leaves. Finally, using a single vine may create the possibility of universal flow failure if the perfusate were to get clogged at any one point in the system.

Conceptual Design 2: Utilization of Leaves and Tubing

In the team's second conceptual design, leaves were considered as the main portion of the plant material that would be used. In this system, the leaves would be connected using sterile tubing. The tubing would provide a system to connect the leaves and provide recirculating vasculature.

The advantages of this design are that the leaves represent the type of vasculature that most closely mimics that present *in vivo*. This is important as it is the main reason our client wanted us to utilize leaves and their nature vasculature. Additionally, the leaves allow for a large surface area in which cells could inhabit. The disadvantages to this design are that the tubing is

not a natural part of the plant which deviates from utilizing all of the natural anatomy available to us. Another disadvantage is that when leaves are perfused, they evacuate the liquid out through the stoma meaning that collecting this perfused liquid with tubing may be difficult.

Conceptual Design 3: Utilization of a Polydimethylsiloxane (PDMS) Mold

The next conceptual design involved the use of a custom-made PDMS mold to encase each individual leaf. The purpose of encasing each leaf is to maintain sterility and also to help facilitate the path of perfusion. The perfusate would travel throughout the entirety of the leaf and the PDMS mold would channel the fluid into the next leaf. This system can be used for any number of desired leaves. The pump keeps the perfusate moving through all the elements without the need for a collection area. Each PDMS mold would encase the leaf with minimal extra space. There would be 3 openings in each mold, one for flow in, one for flow out, and one for air recirculation with a High-Efficiency Particulate Arrestance (HEPA) filter. Each individual PDMS mold, which encases one leaf, would be connected via sterile tubing. Pump-mediated perfusion would be utilized to create a recirculating system.

One advantage of this design is that it controls the directionality of perfusion by using the custom made PDMS mold. Additionally, as the design is composed of multiple sterile chambers, it reduces the chance of contamination.

The disadvantages of this design are that the individual PDMS molds may be difficult to keep sterile if the leaves need to be handled or replaced often. As the leaf chambers are connected in series, it limits the perfusion rate of fluid and may also cause universal failure in the event of a clog. Additionally, there is a limit to the number of leaves the design can support. This is because each leaf would create a pressure drop that would need to be compensated for with an increased pump pressure, potentially destroying the first leaf if the pressure required is too high.

In order to keep the cells on the leaves alive, the media would have to be changed regularly. As there is no collection chamber, this would have to be done very slowly through a needle at a sterile port. This also presents the difficulty of collecting samples for testing.

Feasibility Study 1: Determining the best method of cannulation

Once the leaves are decellularized, they become extremely fragile and movement can compromise the structural integrity of the stem. This is because the needle may poke through the petiole and cause subsequent issues with perfusion and fluid flow. In order to prevent this issue, a feasibility study was conducted to determine the best method of cannulation. Two methods of cannulation were tested. The first method was the silicone glue method. This involved inserting the needle into the leaf via the petiole and then encasing the entire petiole in a silicone glue that was allowed to cure. The second method was the clip method. This involves inserting the needle into the leaf via the petiole, and then encasing the entire petiole in a rigid plastic clip. Both leaves were decellularized via cannulation. Once decellularization was complete, a red dye was perfused via the needle into the leaves. This allowed for observation of perfusion throughout the leaf to observe if the petiole had been compromised during the decellularization process. The set-up and results of this study can be seen inFigure 11.

FIGURE 11. SILICONE GLUE METHOD SET UP, DECELLULARIZATION AND RED DYE TEST

Figure 11 shows the set up for the silicone glue method and the decellularization of the leaf after seven days. The red dye test is also shown and it is clear that only one side of the leaf is being perfused with the red dye. This is due to the fact that the petiole had been compromised by the needle. In the beginning the perfusate would bubble out of the petiole and fall to the bottom of the leaf. However, after 20 minutes, one side of the leaf began to be perfused by the red dye. The results show that the petiole had been compromised and the silicone glue method had failed.

Figure 12 shows the set up for the clip method and the red dye test. The red dye for this leaf is more evenly distributed throughout the leaf and it is clear that the entire leaf would be perfused after a certain period of time. This indicates that the structural integrity of the leaf and petiole remained intact, which proves that the clip method was successful. Based on these results, the team decided to use the clip method for all remaining experimentation with the design and implement the use of the clips into the final design.

FIGURE 12. CLIP METHOD AND RED DYE TEST

Feasibility Study 2: Flow Regulation

For a possible final design, our team examined the idea of having the leaves connected in parallel, rather than in series. In order to allow flow to run in parallel, a manifold was implemented to divide the flow of fluid among all the leaves. The specific manifold that was

used was a polycarbonate individual manifold, with 5 ports and 360° rotation (Cole Palmer). The manifold was used in conjunction with tubing, T-splitters, tubing connectors and pinch valves, if applicable. Tubing was connected to a main collection beaker, which would then connect to a T-splitter where two different pieces of tubing were connected. The two pieces of tubing were then connected to either end of the manifold. This design allowed for a basic setup that could be used as a control to see if there was an even distribution of flow to all of the outlet ports. A representative image of the test set-up can be seen in Figure 13.

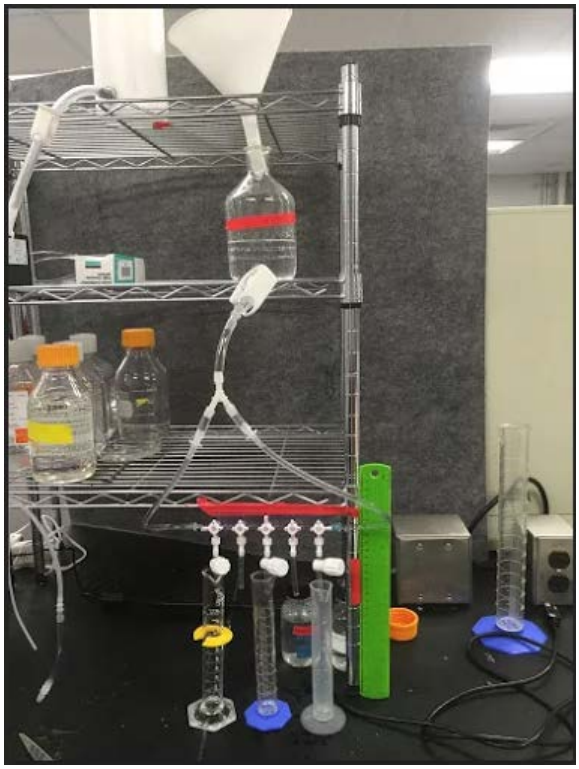


FIGURE 13. REPRESENTATIVE IMAGE OF FLOW REGULATION TESTING SET-UP

The first test that was conducted was a control test. This test consisted of pouring 1000 mL of distilled water into the top beaker and stopping flow to the outlet ports. Then, water was allowed to run through three of the total five outlet ports for 30 seconds. Graduated cylinders were placed below each outlet port to collect the water. Once the 30 seconds passed, the flow of

water was stopped. The amount of water in each graduated cylinder was then recorded and converted into percentages. This was done using the equation: $\frac{observed\ water}{total\ water} \times 100$. This allowed for the calculation of the percentage of water in each beaker so that it could be compared to the expected percentage. This test was completed a total of five times and an average percentage was calculated for each outlet port. Since a three outlet manifold was implemented, a 33.33% distribution was expected in each beaker. As seen in Figure 14, beaker 1 received 30%, beaker 2 received 35.86% and beaker 3 received 34.09% of the total water.

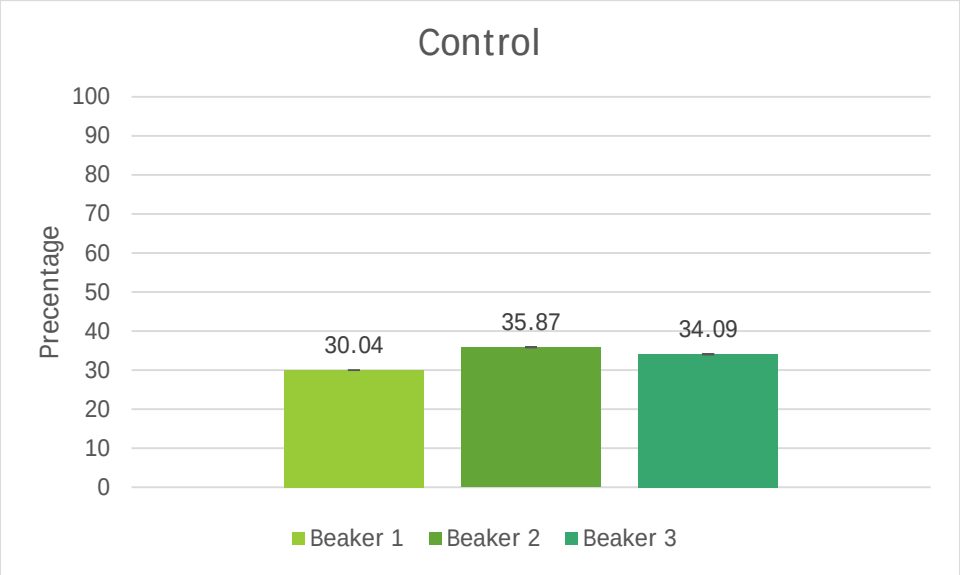


FIGURE 14. CONTROL RESULTS FOR FLOW REGULATION EXPERIMENTS

Although none of these values exactly correlate to 33.33%, they are still statistically significant with a p-value of $8.89*10^{-7}$. Additionally, as this is the control test, this specific distribution of water is important to take into account for the other flow experiments that were conducted.

This control test allows us to run flow in parallel with a working manifold for our final design. However, this specific set-up does not accurately represent physiological function. In the

human body, when blood is pumped from the heart it is not evenly distributed. Each organ receives a different percentage of the total blood, so this must be accounted for in the final design if *in vivo* conditions are going to be represented as closely as possible. A solution to representing this in our final design was the use of pinch valves. Specifically, the design utilizes Flow Control Needle Pinch Valves from the United States Plastic Corporation as seen in Figure 15. These valves can be used to control fluid flow by occluding the tubing. Each clamp has an adjustment range of 10 millimeters (mm) so the occlusion of the tubing can be quantitatively measured.

FIGURE 15. PINCH FLOW VALVES

The tubing that was used for testing would allow the pinch valve to close to 8 mm before contacting the tubing. It was also found through trial-and-error that the pinch valve could close to 4 mm while maintaining an equal flow rate to unrestricted tubing. The difference in flow from the unrestricted tubing was only apparent once the valve was closed to 3 mm.

A single channel test was conducted to see how each pinch valve would control flow without the influence of the other pinch clamps or outlet ports. In order to get a good representation of the flow profile, every half millimeter was tested. 1000 mL of water was

poured into the top beaker and allowed to run through one outlet port with a pinch valve for five seconds. Each setting on the pinch valve was tested five times and the average was calculated. These results can be seen in Figure 16.

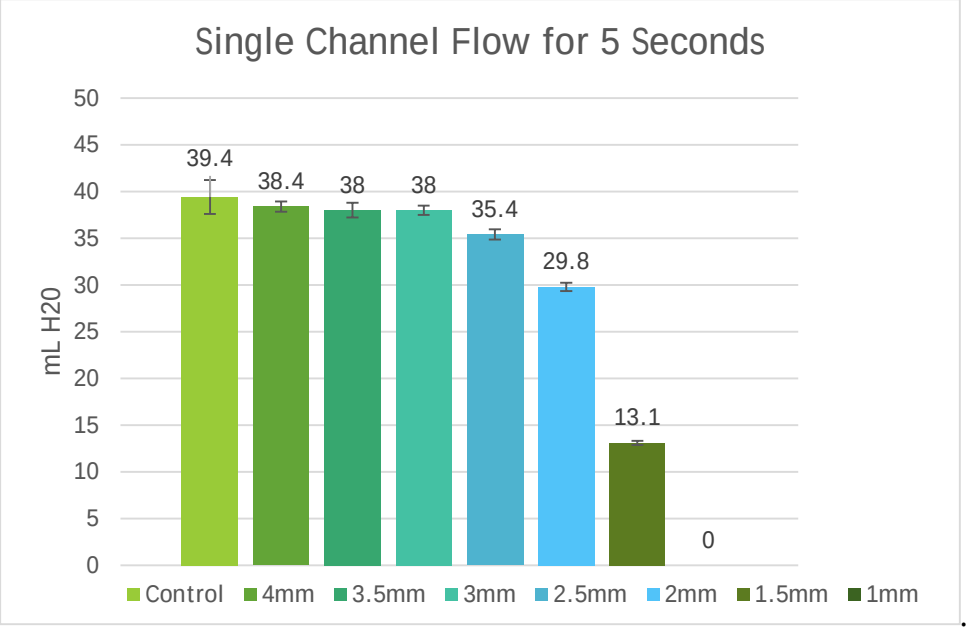


FIGURE 16. SINGLE CHANNEL FLOW RESULTS

By using only one flow channel it was show that the pinch valves are able to regulate the flow, but with very small changes in every half millimeter increment until 2.5 mm. Although this does not show that the pinch valves are effective in regulating flow, it is important to note that multiple pinch valves will be used with multiple outlet ports for our final design. Due to this fact, testing with multiple outlet ports was essential.

As it was expected that pinch valves would be used in conjunction with multiple leaves, a pinch valve was placed on each of the three outlets that were used in the control test. A set up of this testing can be seen in Figure 17.

FIGURE 17. FLOW RESTRICTION TESTING SET-UP

The pinch valves were all set to different restrictions (the largest being 4 mm) in order to determine the ability of the pinch valves to regulate flow. 1000 mL of water was added to the top beaker and the water was allowed to flow for 30 seconds. Each experimental set-up was tested five times and the average for each outlet port was calculated. For one trial, the pinch valves for beaker 1 and beaker 2 were set to be open 2 mm and the pinch valve on beaker 3 was set to be 4 mm open. This indicates that one would expect 25% of the water in beaker 1 and beaker 2 and 50% of the water in beaker 3. The results obtained can be seen in Figure 18.

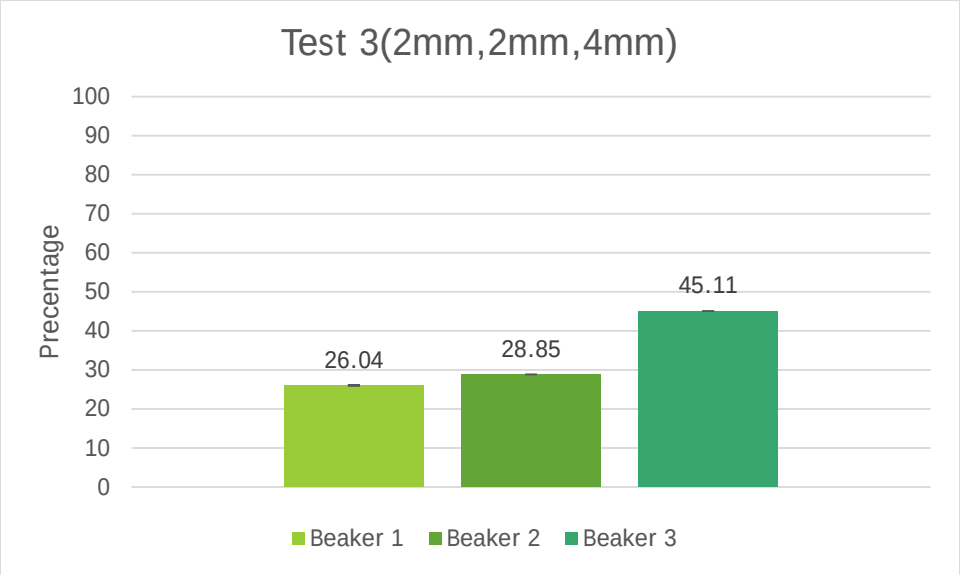


FIGURE 18. TEST 3 FLOW RESULTS

The results that were obtained were that the first beaker got 26% of the total water (compared to the expected 25%), the second beaker got 29% of the total water (compared to the expected 25%), and the third beaker got 45% of the total water flow (compared to the expected 50%).

For a different trial, the pinch valve for beaker 1 was set to 2 mm, the pinch valve for beaker 2 was set to 3 mm and the pinch valve for beaker 3 was set to 2 mm. This indicates that one would expect to see 28.6% of the total water in the first and third beaker and 42.8% of the total water in the second beaker. The actual results that were obtained can be seen in Figure 19.

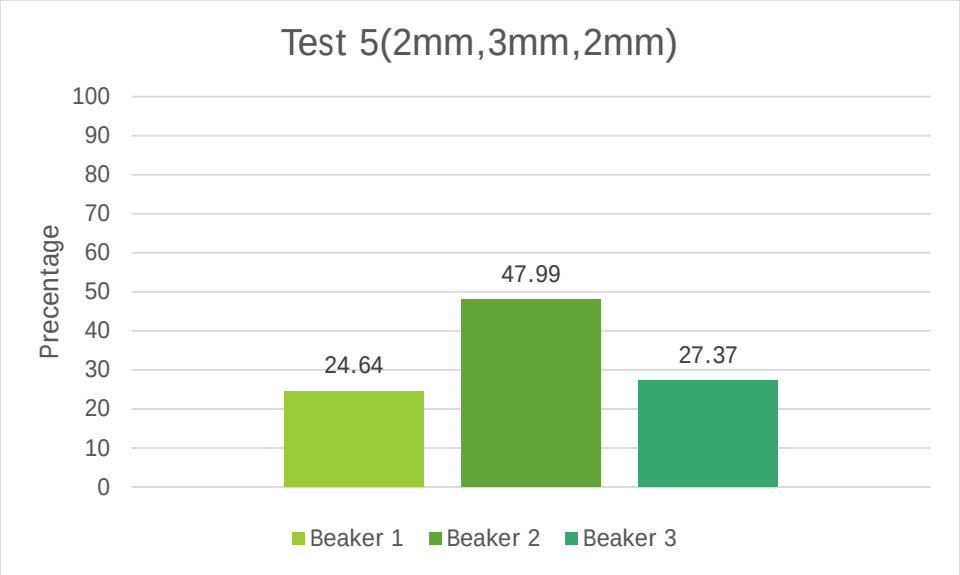


FIGURE 19. TEST 5 FLOW RESULTS

The first beaker received 24.6% of the total flow (compared to the expected 26.8%), the second beaker received 48% of the total flow (compared to the expected 42.8%) and the third beaker received 27.4% of the total flow (compared to the expected 27.4%).

For the final test, the pinch valves for beakers 1 and 3 were set to 3 mm and the pinch valve for beaker 2 was set to 2 mm. This indicates that one would expect to see 37.5% of the total water in the first and third beaker and 25% of the total water in the second beaker. The results obtained can be seen in Figure 20.

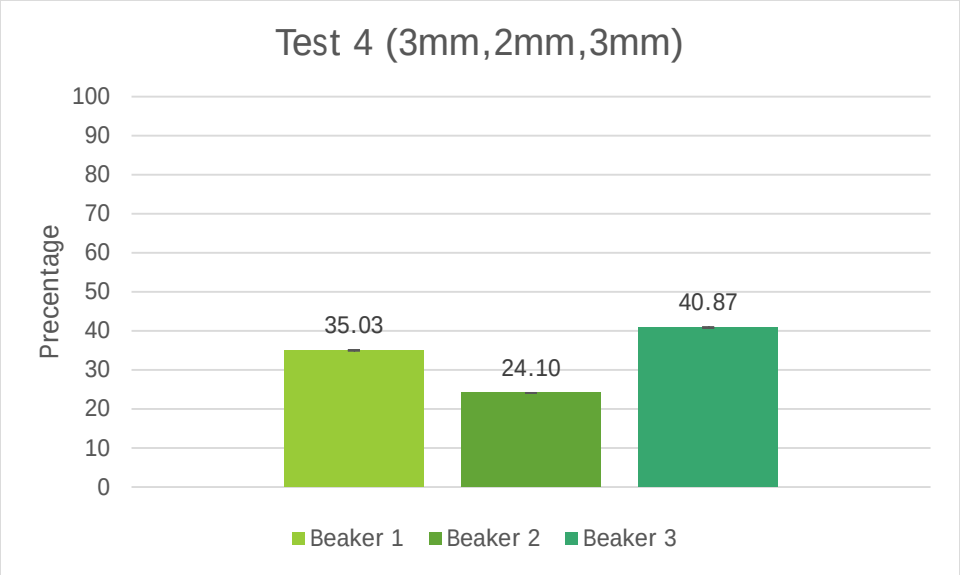


FIGURE 20. TEST 4 FLOW RESULTS

The results were that the first beaker got 35% of the total flow (compared to the expected 37.5%), the second beaker got 24.1% of the total flow (compared to the expected 25%) and the third beaker got 40.9% of the total flow (compared to the expected 37.5%). Overall it is clear that the pinch valves are effective in regulating flow when multiple pinch valves are used in parallel. Additionally, these pinch valves are important as the amount of flow that is regulated can be quantitatively recorded. It does not necessarily matter how much water goes to each outlet, but that the ratio of the water flow to each different outlet port can be controlled in order to model *in vivo* conditions.

Feasibility Study 3: Pressure Regulation

To achieve a more compact device with leaves resting horizontally, and to maintain consistant perfusate flow through the leaf, a pump mediated pressure regulator was created. An Arduino microtcontroller, which receives data from a pressure transducer, controls a parastaltic pump. To assess this regulation system's capability of maintaining a constant pressure within the leaf, data was collected under two different conditions. Condition one involved the pumping of

fluid through an open tube with no leaf attached, while the second condition involved pumping through a leaf. The pressure measurements for both setups can be seen in Figure 21 and Figure 22. Through the electronically regulated pressure fluctuates more, this fluctuation is controlled and due to the pulsatile nature of the peristaltic pump. The fluctuations in pressure recorded from the mechanical pressure head are not controlled and sue to its passive nature is prone to becoming clogged. These results indicate that it is possible to develop a compact system that actively pulses perfusate within a specified range.

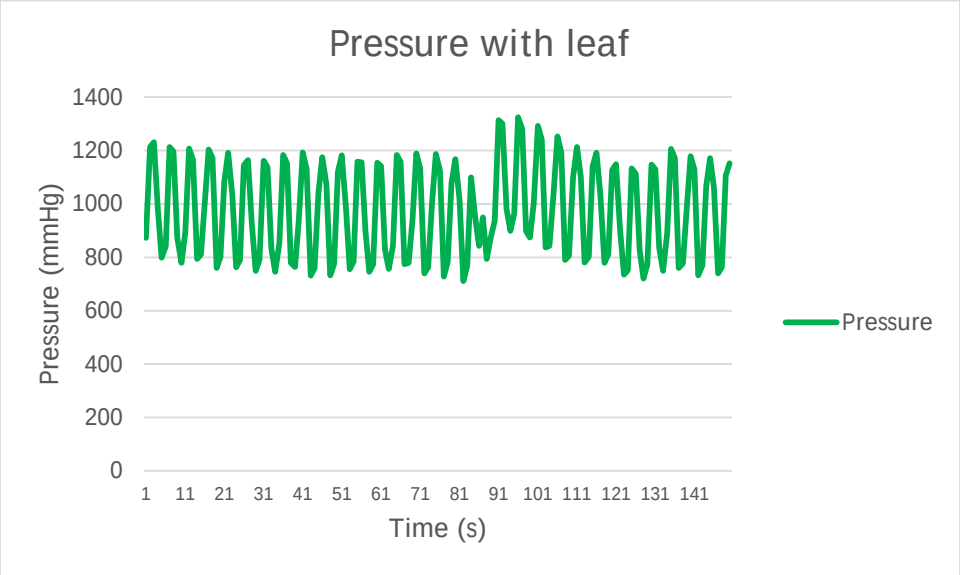


FIGURE 21. PRESSURE WITH LEAF

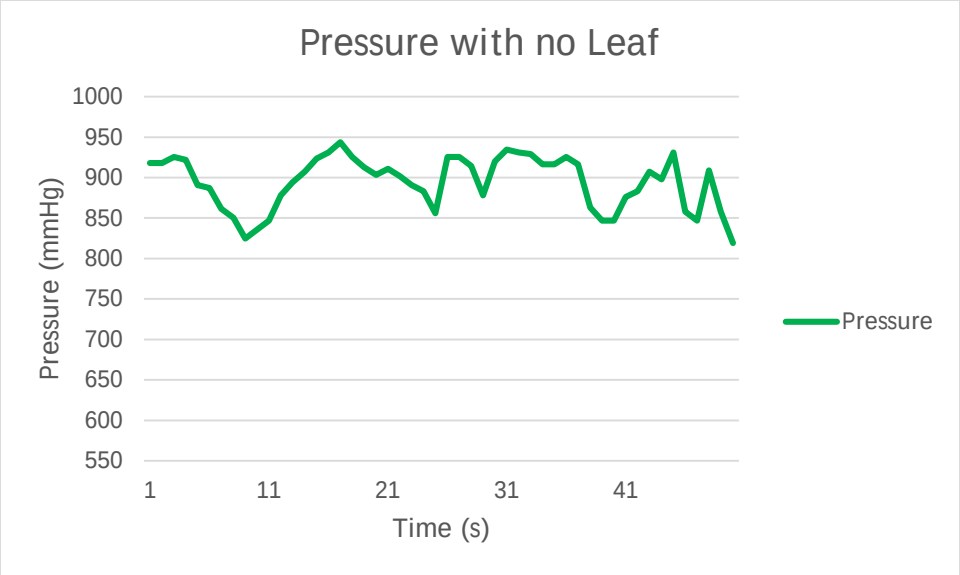


FIGURE 22. PRESSURE WITH NO LEAF

4.3 Alternative Designs

The team established various alternative designs that would allow for both the decellularization and cell seeding of plant material with recirculating vasculature. These differ from conceptual designs in that they are more detailed and their mechanical set-up is very specific. It was essential that these alternative designs also have the capacity to achieve all of our defined objectives, constraints and functions. Descriptions of these alternative designs are provided in the following sections along with each designs advantages and limitations.

Pump mediated perfusion system of stems placed in series

The first alternative design utilized stems as the main portion of the plant material that would be used as seen in Figure 23. Tubing would be arranged in a circle so that the perfused liquid could continuously move through all of the stems. Additionally, a pump would be used to create flow which could flow in either direction. This design would not require a collection bin as the perfusate would be contained within the stem pieces or tubing. Tubing would be arranged in a circle so that the perfused liquid could continuously move through all of the stems. Additionally, a pump would be used to create flow which could flow in either direction. This

design would not require a collection bin as the perfusate would be contained within the stem pieces or tubing.

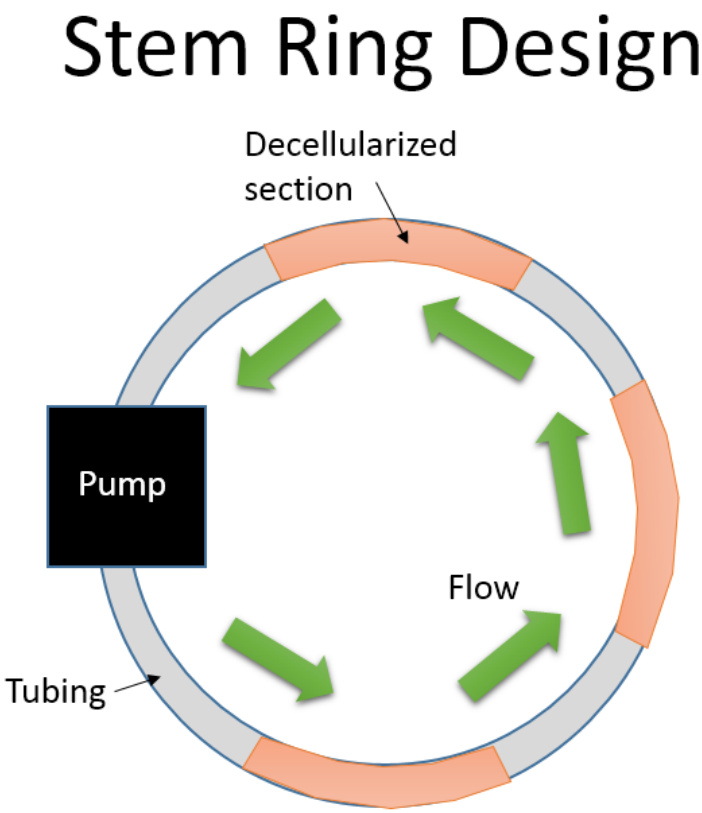


FIGURE 23. PUMP MEDIATED PERFUSION SYSTEM OF STEMS PLACED IN SERIES

The advantages of this design are that the stems would allow for recirculating vasculature without the liquid being evacuated out by the leaves. Another advantage is that the stems would provide a clear path of recirculating media which could easily be controlled by pump driven perfusion. Lastly, this is a simple design that would be small and easy to build.

The disadvantages of this design are that the stems may be difficult to decellularize, as they are more robust than the leaves they are attached to. Additionally, using stems does not utilize the natural vasculature of plants which mimics *in vivo* conditions and thus does not satisfy the client's motivations for the final design. Another disadvantage of this design is that it is

limited to the stem only, which only utilizes a small portion of the plant anatomy available to us. The final disadvantage of this design is that it does not allow for variable flow between the different stem pieces.

Gravity and pump mediated perfusion system of *horizontally* oriented leaves placed in series

The next alternative design was a gravity and pump mediated perfusion system of horizontally oriented leaves placed in series as seen in Figure 24. This design incorporates gravity mediated perfusion in which the leaf lays horizontally on an acrylic board. This acrylic board has perforated holes to allow fluid flow from the bottom of the leaf to the lower collection chamber. The bottom collection is connected to a tube which facilitates fluid flow from the collection chamber to the next leaf in the series. The system will maintain sterility as the leaves will be covered with a clear acrylic top for each collection chamber. The tube is connected to a needle which is inserted to the stem of the next leaf. This fluid then passes through the vasculature of the leaf to allow for full perfusion of the leaf, until the fluid is sweated out by the leaf and the liquid falls in to the lower collection chamber. This system continues for the desired numbers of leaves. The individual units are placed on top of each other so that gravity can mediate the movement of fluid from one leaf to the next. The collection chamber of the bottom most unit is connected to tubing which will connect back to the top most leaf. This specific movement of fluid will be assisted with the use of a peristaltic pump as the fluid will be moving against the direction of gravity.

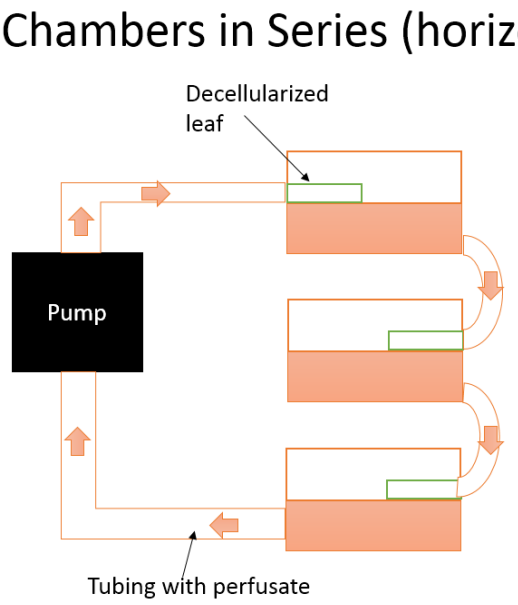


FIGURE 24. GRAVITY MEDIATED PERFUSION SYSTEM OF HORIZONTALLY ORIENTED LEAVES PLACED IN SERIES

One positive attribute of this design is that the design is compact. This will allow the team to easily handle the system when transferring it from the incubator to the sterile hood. Another positive attribute of the design is that the design can be one or many chambers. This is useful for testing purposes as initially fewer leaves can be used to validate the design and additional leaves can be added later for experimental purposes. This design is mostly gravity driven versus the use of a peristaltic pump for each leaf which minimizes the cost of design. Another positive attribute of this design is that it satisfies all of our defined design objectives.

The first negative attribute is that it is very possible for universal failure to occur with this design. This means that if one part of the system were to be non-functioning, i.e. one section of tubing was clogged, then the entire system would fail. Another negative attribute to this design is that it is difficult to control the flow between each leaf. Although a gravity driven system is a positive attribute, it can also be seen as a negative attribute as it does not allow for fluid flow variability or control by the user.

Gravity and pump mediated perfusion system of *vertically* oriented leaves placed in series

The final alternative design is a gravity and pump mediated perfusion system of vertically oriented leaves places in series as seen in Figure 25. This design incorporates the leaves where the perfusate is passed from one leaf to another in series. Each leaf would be encased in its own sterile unit which would help to avoid contamination. Initially, it was thought that the leaves could be connected in series with one set of tubing. It was concluded that if the first leaf in the series failed, it could potentially stop the flow of media to the rest of the leaves. If media did not flow through all of the leaves, the cells on the leaves could possibly die. Taking possible test failures and blockages into consideration, our team designed a new system that would allow for flow in the event of test failures and blockages.

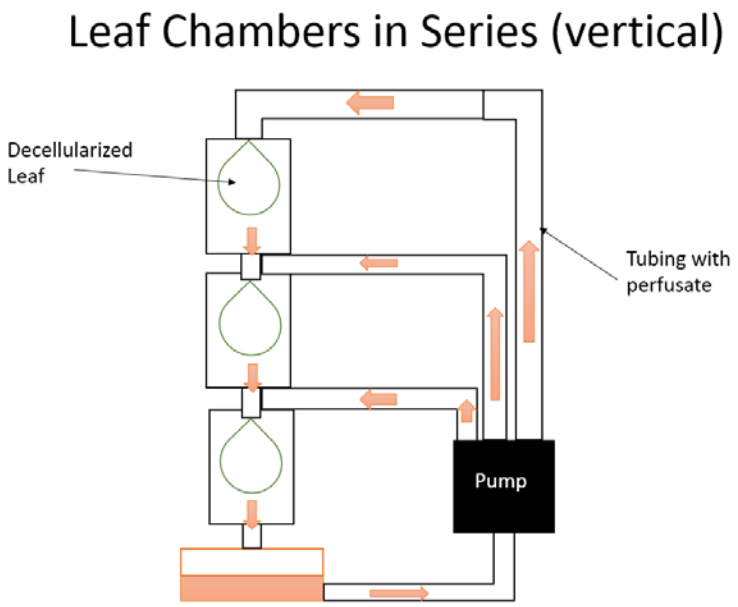


FIGURE 25. GRAVITY AND PUMP MEDIATED PERFUSION SYSTEM OF VERTICALLY ORIENTED LEAVES PLACED IN SERIES

To allow flow to different test subjects, a tubing system was designed. The media would be stored in a single collection bin that would feed into a pump. There would be a main tube that

would allow flow into the first leaf, and from the first leaf, the media would flow to the next leaf and so on. The pump tubing, however, would have splits in it leading to each of the other leaves to allow for continuous flow to all of the leaves. This would allow for failure in the first leaf to be bypassed and continue flow to the other leaves so universal failure would not occur.

The advantages of this design are that it satisfies most of the design objectives such as, facilitates plant decellularization, has multiple cell types on multiple leaves and models active vasculature using plant anatomy. Another advantage to this design is that it has fail safe tubing, as described earlier which also allows for the possibility to control flow between each of the leaves. Finally, this design is conceptually simply meaning that the design and fluid flow is easily understood and not overly complicated.

One disadvantage of this design is that the plant size is limited by the size of the bottles, which would be used to contain the leaves. The leaves would also be oriented vertically. In this position the structural integrity of the stem is risked. Along with this, another disadvantage is that each individual leaf would need its own bottle, which would require a lot of unnecessary materials. Additionally it would be impossible to seed cells onto the leaves without removing them from their bottles. This could be a potentially risky and difficult process. The last disadvantage of this design is that is large and would take up a lot of space in the incubator or sterile hood. It would be difficult to transport and move between the incubator and sterile hood, which is essential for any type of cell and tissue culture.

4.4 Final Design Selection

To determine the final design, a Pairwise Comparison Chart was created, as seen in Table 4, to compare each of the alternative design's and our final chosen design's abilities to satisfy our objectives. Each design was scored based on how well it would meet each objective. From

our analysis, we deemed the pump mediated perfusion system of leaves placed horizontally in parallel to be the best suited design.

TABLE 4: PAIRWISE COMPARISON CHART

Design	Objectives If meats criteria then it will receive a 1, if it doesn't meet criteria then it will receive a 0. If only meet requirement partial then it will receive a .5.				
	Able to facilitate decellularization	Able to facilitate cell seeding	Able to host multiple cell types on multiple leaves	Provide stable extracellular substrate	Protect structural integrity of the leaf
Pump mediated perfusion system of stems placed in series	0	1	1	0	0
Gravity and pump mediated perfusion system of *horizontally* oriented leaves placed in series	1	0	1	1	1
Gravity and pump mediated perfusion system of *vertically* oriented leaves placed in series	1	1	1	1	0.5
Pump mediated perfusion system of leaves placed *horizontally* in parallel	1	1	1	1	1

This chosen design is a modular device, made of acrylic, which can fit in an incubator and can be sterilized using ethylene oxide. There are two main components to this design, a chamber where the leaves are kept and an electronics chamber to regulate continuous flow. A schematic of the design can be seen in Figure 26.

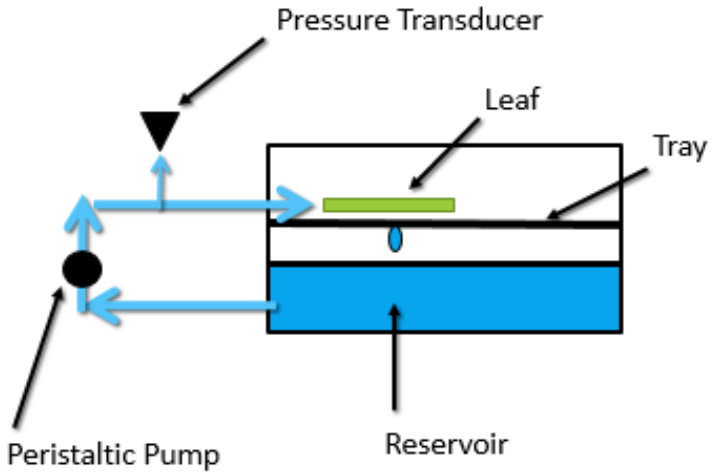


FIGURE 26. SCHEMATIC OF CHOSEN DESIGN

The leaves are place on an acrylic sheet that has small holes to allow the perfusate to fall through into a collective reservoir. A peristaltic pump is used to transport the perfusate in this reservoir back into the leaves through tubing and a manifold which divides perfusate flow. The pump also cycles a small amount of perfusate to a pressure transducer that is controlled by an Arduino board which regulates the pressure. There are also two fans, one incoming and one outgoing, to regulate heat within the electronics chamber. One fan pulls room temperature air from outside of the incubator, through tubing, into the electronics chamber. The other fan pushes the warm air from inside of the electronics chamber, through tubing, to the outside of the incubator. Since these electronics with be in an incubator, they are coated with an electronic grade coating spray which creates a hydrophobic surface, allowing the electronics to function in the humidity.

In prior literature, decellularization has been achieved by elevating the reservoir of perfusate above the tissue sample of interest, in order to create gravity driven perfusion. This creates a relatively constant pressure that pushes the perfusate through the vascular network of

the tissue. Though this setup is sufficient for decellularization purposes it is not ideal for a system that must support the growth of cells, due to the fact that it must fit into an incubator. In order to fit into an incubator the system must take on a more compact design.

The pump mediated perfusion system of leaves placed horizontally in parallel system was the only design to incorporate all of the design objectives. Since the tubing goes to each leaf simultaneously and it has a collective reservoir, it can be used for decellularization as well as cell seeding. By making this a two-in-one system, the leaves are not harmed in transport from a decellularization device to a system for cell seeding. This is important in maintaining the structural integrity of the leaves as decellularized leaves are very fragile and at risk of being compromised if they are moved frequently. This system is best able to maintain the structural integrity of the leaves because of the tray which provides a flat surface for the leaves to rest on.

In order to mimic physiological function, multiple leaves can be utilized to house multiple cell types within our system. The recirculating perfusate in our system will allow for cell type to cell type interactions. In order to maintain cell viability, it is important that the system is able to be sterilized and maintain a sterile environment. The system was sterilized using ethylene oxide. System sterility was tested by placing media in the reservoir and incubating it for two days, with media in all of the tubing. After two days, the media was examined under the microscope. No growth was found and the system was declared sterile. Having met all the design objectives, the pump mediated perfusion system of leaves placed horizontally in parallel was chosen as our final design.

V. Design Verification

There are many aspects of the final design that had to be tested in order to ensure that the best methodologies were being employed to meet our design objectives, constraints and functions. This section presents in detail the tests that were performed and the results of each of these tests. This chapter will help to further understand the final design choices that were made and the supporting verification of those decisions.

5.1 Functionality in the incubator

It is imperative that the system is able to operate inside of an incubator to maintain the viability of the cells. The environment that the incubator provides is a hostile environment for the electronics that regulate the flow of the perfusate. As the inside of the incubator is 37 degrees Celsius and has a humidity above 80%, electrical components may experience corrosion and shorting of the circuit may occur. Measures were taken to protect the circuit and its components from the harsh environment inside the incubator. These measures include a hydrophobic coating for the electrical components and fans with intake and outtake tubing from the sealed electronics chamber.

The primary means of protection for the electronic components is a hydrophobic electronic grade coating that covers each electronic component. This coating acts as a physical barrier between the electronics and the humidity in the air.

As a secondary means of protection, two small fans were installed in the electronics chamber with large diameter tubing leading outside the incubator. These fans exchange the humid air from inside the electronics chamber for dryer air outside of the incubator. These fans also prevent the electrical components from overheating by providing cool dry air to the chamber.

5.2 Successfully decellularizing plants

One of the objectives that was originally established for this system is that it should facilitate the decellularization of multiple leaves. As previously stated the standard method of decellularization involves inserting a cannula into the petiole of the leaf and suspending it vertically. An elevated reservoir is then connected to that cannula and passively circulates the perfusate through the leaf's vasculature using gravity. The appearance of the leaf changes throughout the decellularization process. The leaf changes from its normal opaque green appearance to a translucent green. From there, the leaf loses its color and assumes a clear translucent appearance.

Our system aims to replicate the decellularization quality of the standard method by actively circulating the perfusate through the vasculature of the leaf using a peristaltic pump. The peristaltic pump is controlled by an Arduino microcontroller which adjusts the speed of the pump in order to maintain a constant pressure. A notable difference between the two methods is that the leaf assumes a horizontal orientation rather than a vertical one. However, the sequence of detergents used for decellularization remains the same.

To test the system's ability to decellularize, leaves were connected to three of the four available ports. The last port was sealed for sterilization purposes. All of the leaves rest in a horizontal position on the tray. The protocol used for decellularization can be found in Appendix A: Protocol for Plant Decellularization. The first detergent was loaded into the reservoir and the Arduino was activated. This Arduino has been programmed using the coding found in Appendix F: Software Code for Pump. Once activated the Arduino started the pump with an initial velocity of 150 revolutions per minute (rpm). Once the perfusate advanced to the pressure transducer, the Arduino began to adjust the speed of the pump to control the range of pressure variation. The

perfusate was then free to advance to the manifold where it was divided among the ports. As each leaf was perfused, the perfusate was evacuated through the stomata of the leaf and passively returned to the reservoir through the holes in the tray.

After each step of the decellularization process, the reservoir was emptied and refilled with the next solution. The progress of the decellularization was visually assessed throughout the process. The physical appearance of some of the leaves in our system can be seen in Figure 27. To prevent contamination of its contents, the user avoided waiving their hands over the open chamber and making direct contact with the interior surfaces of the chamber.

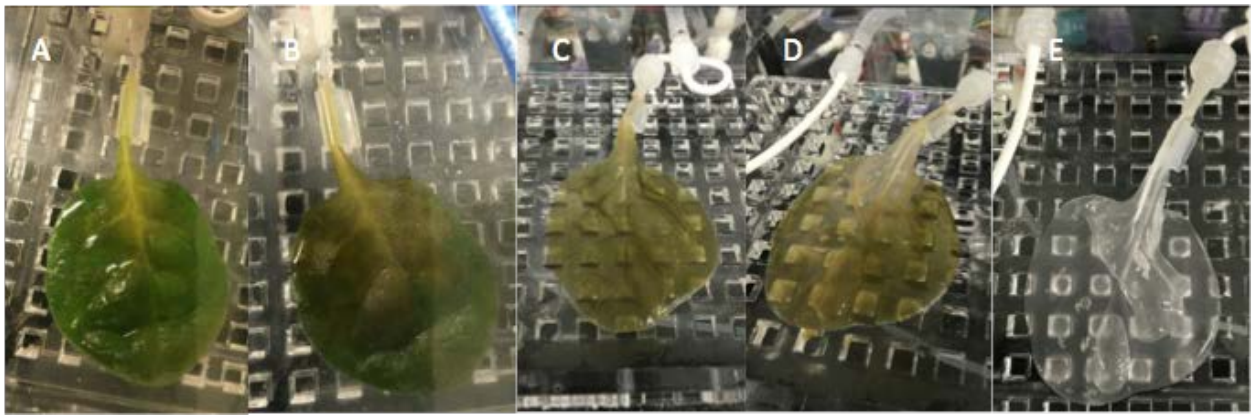


FIGURE 27. DECELLULARIZATION PROCESS USING OUR SYSTEM

Once the leaves appeared to be completely decellularized, it was histologically confirmed. The leaves were processed, embedded in paraffin wax, and cut to a thickness of 8 µm using a microtome. A Safranin O and Fast Green stain was used to compare our decellularized leaves with those decellularized using the current system. The protocol for this stain can be found in Appendix C: Protocol for histological stain for cell wall and cytoplasm. These histology results can be seen in Figure 28.

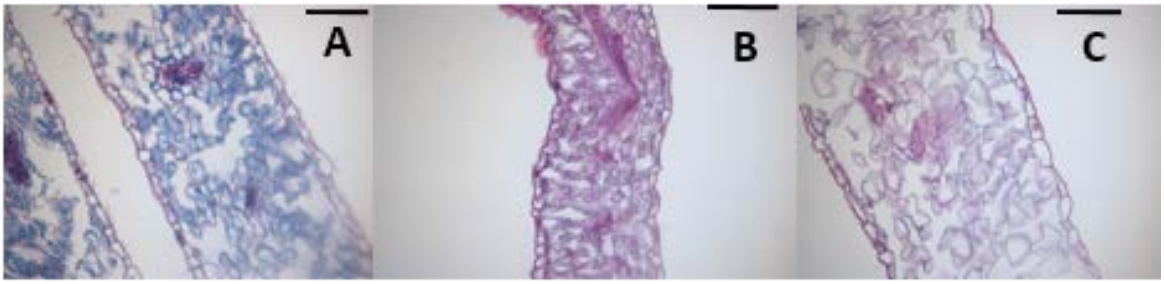


FIGURE 28. SAFRANIN O AND FAST GREEN STAIN OF THREE PIECES OF SPINACH AT 20X MAGNIFICATION A) SPINACH CONTROL B) DECELLULARIZED SPINACH USING CURRENT GOLD STANDARD C) DECELLULARIZED SPINACH USING OUR SYSTEM (BLUE=CELL MATERIAL, PURPLE=CELL WALL)

As seen in Figure 28A the spinach control has blue coloration which indicates that cellular material is present. Figure 28B and Figure 28C are decellularized spinach. The absence of blue coloration in these images confirms that there is no cellular material present. Figure 28B is decellularized spinach using the current gold standard. Figure 28C is decellularized spinach using our system. The absence of blue coloration confirms that our system successfully decellularized the spinach leaf.

5.3 Seeding Cells onto Leaves and Perfusion Testing

A main function of our design is to seed cells onto the leaves and allow them to grow. It was necessary to confirm that cells could remain in place on the leaf. To test this, a protocol was used that is frequently implemented in the Gaudette Laboratory and can be seen in Appendix B: Plant Co-culture Protocol.

To test the system's ability to facilitate cell seeding, a sterile decellularized leaf was seeded in our system with quantum dot loaded human mesenchymal stem cells (hMSCs). Quantum dots are nanoparticles that excite under a narrow UV range. The cell seeding process can be found in Appendix B: Plant Co-culture Protocol. After the cells were successfully placed

on the leaf they were allowed to grow for 24 hours while being perfused with media. The cells were imaged using fluorescent microscopy. The resulting images are found in Figure 29.

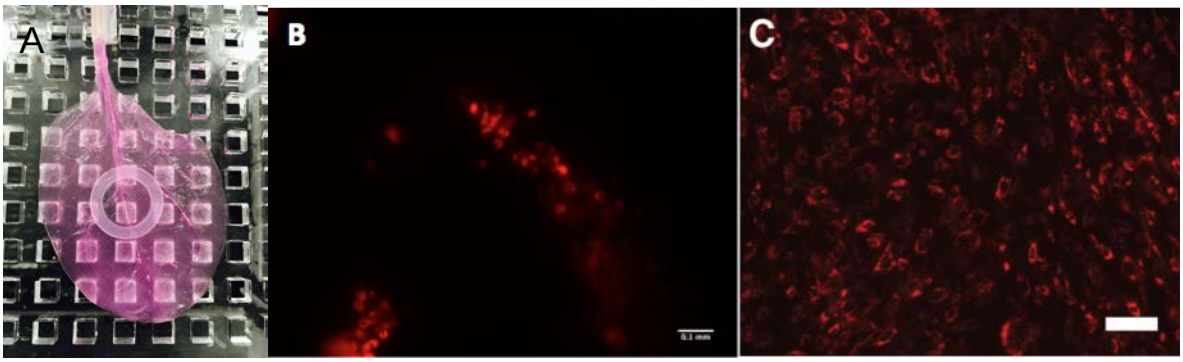


FIGURE 29. A) CELL SEEDED LEAF IN THE STERILE HOOD B) FLUORESCENT MICROGRAPH IMAGE WITH ACTIVATED QUANTUM DOTS OF CELLS SEEDED ON A LEAF C) FLUORESCENT MICROGRAPH IMAGE WITH ACTIVATED QUANTUM DOTS OF CELLS SEEDED ONTO A FLASK

Figure 29A is an image of a leaf seeded with cells in our system. Figure 29B is a fluorescent micrograph image of a leaf seeded with cells. Figure 29C is a fluorescent micrograph image of a flask seeded with cells. Due to the presence of quantum dots in Figure 29B, it can be confirmed that the cells were successfully seeded onto the leaf.

5.4 Cell Viability

In addition to testing the ability to seed cells onto the leaves, it was also necessary to determine that the cells could survive media perfusion. This is essential because the final design will perfuse media through the petiole in order to create a recirculating system while also maintaining cell viability. Cells were seeded onto a leaf as described in Appendix B: Plant Co-culture Protocol. The leaf is stained with ethidium homodimer-1 which places a red label on the nucleus of dead cells. Phalloidin and Hoechst were used as counter stains to label the cytoskeleton and nuclei respectively. As a result all cells will appear to have green bodies with nuclei present. Live cells will appear to have blue nuclei, whereas dead cells will appear to have purple nuclei. The purple color is due to the overlay of the blue and red from the Hoechst dye

and the ethidium homodimer-1. This protocol can be found in Appendix D: Protocol for live/dead stain. The resulting image can be seen in Figure 30.

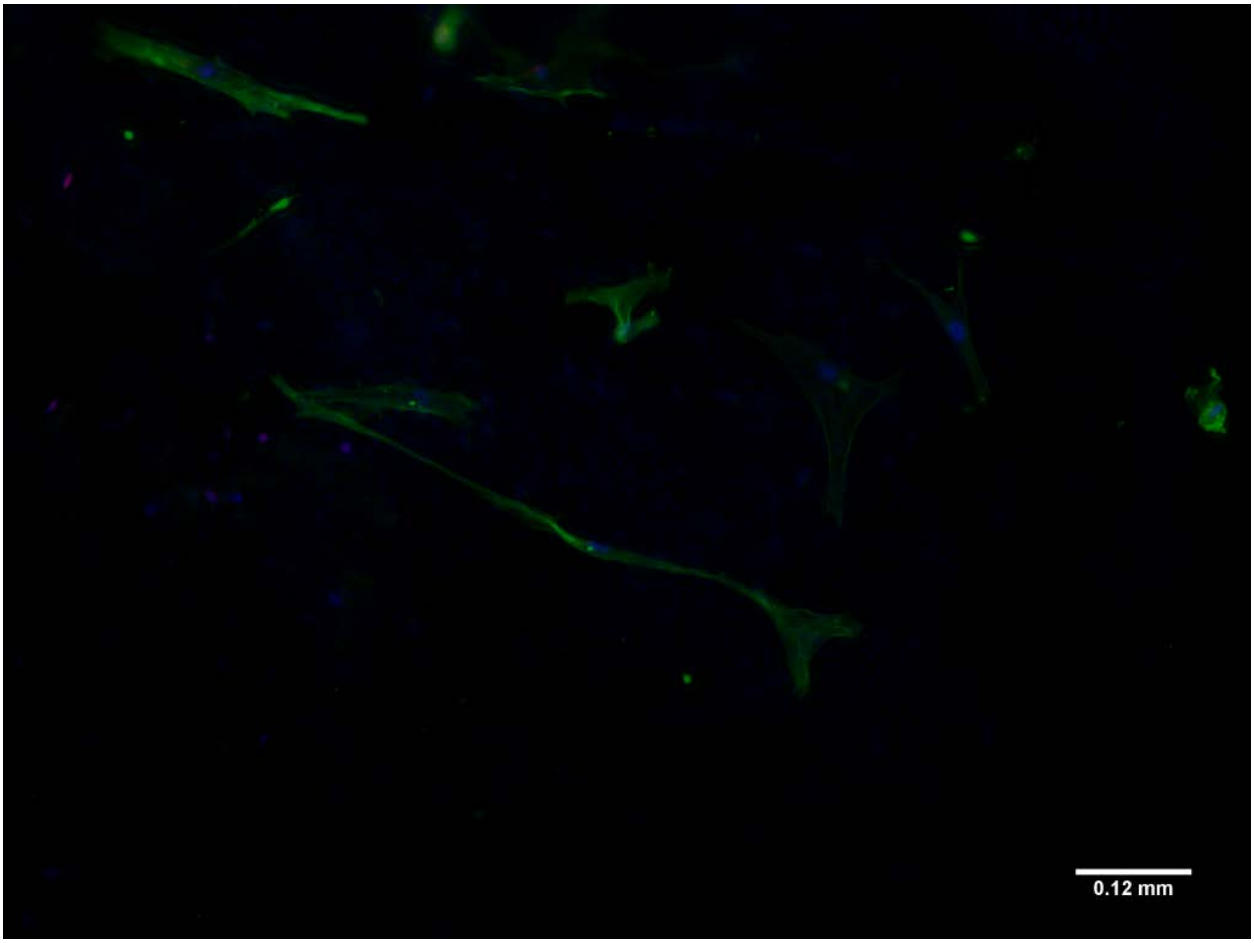


FIGURE 30. ETHIDIUM HOMODIMER-1 (RED), PHALLOIDIN (GREEN), AND HOECHST (BLUE) STAIN OF SEEDED LEAF

There are blue cells present in Figure 30 with no red color overlaying them. This indicates that cells were viable on the leaf after 24 hours.

VI. Final Design and Validation

6.1 Final Design Description

The final design of the pump mediated decellularization and cell seeding system consists of two acrylic chambers. These chambers were drafted in Solidworks and assembled using acrylic sheets and acrylic cement. The first chamber hosts a reservoir for fluid agents and a horizontal support tray on which the cannulated leaves rest as seen in Figure 31. This chamber's top side is attached to a hinge allowing easy access to the fluids, leaves and cannulas for preparing the system. This will also allow the user to seed cells onto the plants. The second chamber houses an Adafruit peristaltic pump, power source, pressure transducer, two fans and an Arduino microcontroller as seen in Figure 32. The first fan draws cool, dry air from outside of the incubator into the electronics chamber using tubing and a second fan expels the warm air from the chamber outside the incubator using tubing. Silicone tubing is used to connect both chambers and a manifold is used to divert fluid flow.

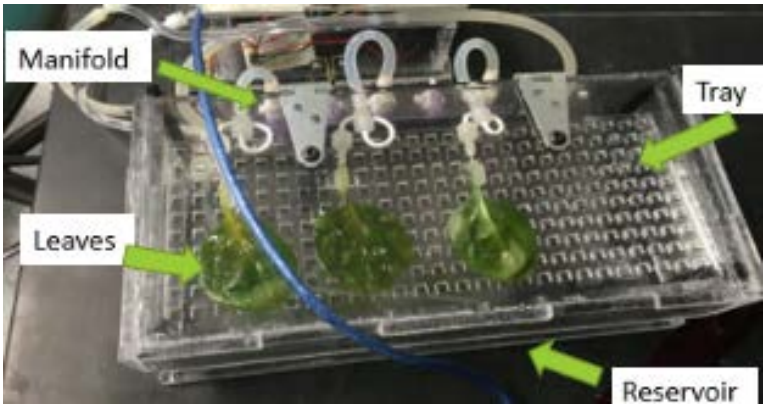


FIGURE 31. CHAMBER 1 WHICH HOSTS A RESERVOIR FOR FLUID AGENTS AND A HORIZONTAL SUPPORT TRAY ON WHICH THE CANNULATED LEAVES REST

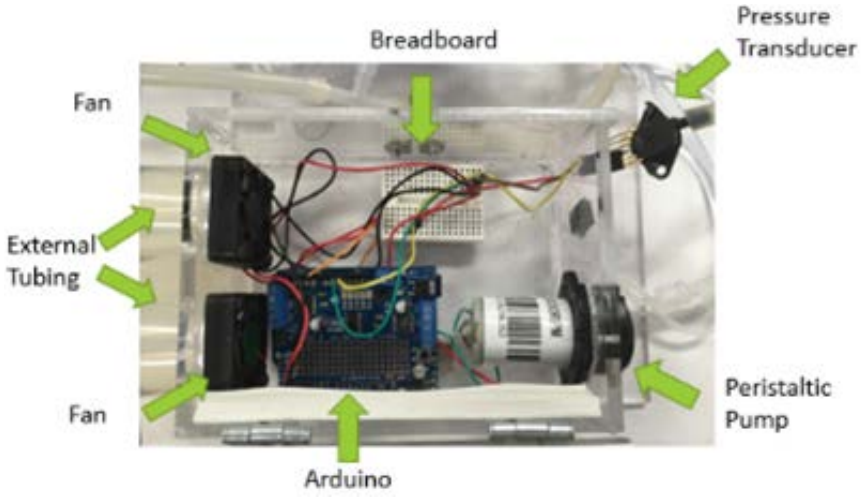


FIGURE 32. CHAMBER 2 WHICH HOSTS AN ADAFRUIT PERISTALTIC PUMP, POWER SOURCE, PRESSURE TRANSDUCER, 2 FANS AND AN ARDUINO MICROCONTROLLER

Chamber one is completely detachable from the system and has been designed to be sterilized via ethylene oxide. Detachable silicone tubing is fixed to the bottom of chamber one's reservoir through which the fluid agents are pumped. The fluids enter chamber two and reach a transducer which collects pressure data. This data is translated by the Arduino microcontroller which has been programmed to control the rate of the pump based on this pressure data. The fluids exit chamber two and reaches a manifold where flow is divided into five separate ports. The fluid in each port then reenters chamber one and is pumped through the cannulated lumen of a leaf where it is perfused throughout the microvasculature. The fluid then exits the leaf through the stomata and returns to the reservoir for recirculation. The system's fluids are changed throughout the process in accordance with the decellularization and cell seeding protocols found in Appendix A: Protocol for Plant Decellularization and Appendix B: Plant Co-culture Protocol respectively. The process in which the design functions is visualized in Figure 26.

6.2 Achieving the objectives

Our client was seeking a systems biology device which involves the decellularization and cell seeding of leaves within the same perfusion system to form a biomimetic body that mimics

physiological systems *in vivo*. To do this, we identified, addressed, and tested five crucial objectives for our design.

1. Protect the structural integrity of the plant

The design must protect the structural integrity of the leaf in order to ensure that perfusion occurs properly, for in the event that the structural integrity is compromised incomplete decellularization or cell death may occur. To achieve this objective cannulation methods were analyzed using a red dye perfusion test for their ability to protect the leaf's vasculature as described in Feasibility Study 1: Determining the best method of cannulation. After choosing the most effective method, which was the clip method, a horizontal tray was also incorporated on which the leaf may rest to prevent motion of the leaf.

The ability to protect the structural integrity of the leaf was proven following successful decellularization attempts using the device. In the event that the leaves' structural integrity became compromised, full decellularization would not have been possible.

2. Able to facilitate plant decellularization and cell seeding

The design must facilitate the decellularization and cell seeding of plants. In order to model the human body, plant cells must be removed and human cells must be seeded onto the leaf. To achieve this objective our device was outfitted with the capabilities of executing the decellularization and cell seeding protocols found in Appendix A: Protocol for Plant Decellularization and Appendix B: Plant Co-culture Protocol respectively through the use of our fluid reservoir and pump mediated perfusion system.

We assessed the efficacy of decellularization by using a histological stain that stains for cell wall and cytoplasm. This was done by processing the leaf, embedding it in paraffin wax, cutting the leaf via microtome and using Safranin O and Fast Green stain (protocol can be found

in Appendix C: Protocol for histological stain for cell wall and cytoplasm. Images were analyzed qualitatively and observed to show that the samples were adequately decellularized.

We assessed the efficacy of cell seeding by loading cells onto the leaves with quantum dots. Images were analyzed qualitatively and observed to show that cells were successfully seeded onto the decellularized plants.

3. Ability to host multiple cell types on different leaves.

The system must incorporate different cell types that are physically isolated from one another. Different cell types are needed to model the complex human body and the interactions that occur between cells *in vivo*. The cellular communication can then be used as in "*in vitro* model for drug screening" as stated in the client statement. This objective was addressed using a manifold which allowed us to create up to four organoids living on the same interconnected system. Cells from one leaf may produce a metabolite that will be released into the reservoir, where it is mixed in with the complete media and pumped to the other organoids.

We assessed our ability to host multiple cell types on different leaves by seeding cells onto a leaf and using a live/dead assay. Phalloidin, Hoechst, and ethidium homodimer-1 were used in conjunction with one another to obtain a fluorescent image confirming that cells were alive at the after 24 hours on a leaf as seen in Figure 30. By determining that cell viability was maintained on one leaf, we can infer that multiple cell types can survive on multiple leaves in our system.

4. Model active microvasculature using plant anatomy

The design must model active vasculature using plant anatomy as the current gold standard of drug screening currently lack this ability. The main premise of our design involves the use of decellularized leaves' microvasculature as a means of delivering nutrients and drugs to

cells seeded onto them. By successfully building a system that maintains the viability of cells seeded onto a plant via perfused media we have modeled the active microvasculature using plant matter.

5. Provide stable extracellular substrate

The design must allow for a stable extracellular substrate. Once decellularized, the plant matter becomes very fragile and is at a great risk for damage. The design must ensure that the leaf is stable and protected. Any damage to the leaf may cause harm to the cells seeded onto the leaf.

The ability to protect the structural integrity of the leaf was proven following successful decellularization attempts using the device. If the leaves' structural integrity became compromised, full decellularization would not have been possible.

6.3 Design Standards

It is important that our device, testing procedures, and maintenance procedures satisfy each ISO requirement. Section 3.7 of ISO 13485:2003 characterizes our plant based profusion system as a medical device in that it "provides information for medical purposes by means of an in vitro examination of specimens derived from the human body". Because this device is a novel means for screening drugs, there have not been any ISO standards written for this specific device. Following further production, our decellularization and cell seeding device will need to be addressed by ISO and the FDA regarding proper use and whether or not it can be used as a suitable substitute for the conventional methods of drug screening.

6.4 Economics

Our client was seeking a new device for preclinical drug screening. Every year pharmaceutical companies spend billions of dollars completing preclinical and clinical studies in order to achieve FDA approval and enter the market. It is financially devastating when a

company wastes hundreds of millions of dollars on a compound only for it to fail in the late stages of clinical trials. We believe that the technology we researched, developed, and trialed can prevent compounds likely to fail from entering very expensive clinical trials by providing the researchers with a more accurate preclinical testing model. Consequently, the economic impact of this device is significant as it can overcome the limitations of the current gold standard for *in vitro* preclinical drug screening and become a marketable solution for pharmaceutical companies.

6.5 Environmental impact

Our device which was researched, developed and manufactured is expected to have very little environmental impact. All material components including acrylic walls, silicone based tubing, Arduino microcontroller, manifold, fans, copper wiring, cannulas, clips, batteries and spinach leaves are all common materials that are either permanent fixtures, recyclable, or very safely disposable. The potentially harmful chemicals required for decellularization and cell seeding including hexanes, 10% SDS, 0.1% Triton-X 100, 10% bleach, PBS and cell culture media are to be used only within a laboratory setting that is equipped with the proper means of disposal.

6.6 Societal Influences

It is not expected that our device will be recognized by the average citizen. However, there are major societal implications within the pharmaceutical, educational, and scientific communities. This technology features decellularized plants, a novel concept to the field of tissue engineering. This approach could potentially inspire an entire new field of cross-kingdom regenerative engineering. A massive field expansion will inspire new novel devices, treatments, and mindsets to impact and save countless lives.

6.7 Political ramifications

The political and legal implications of the technology developed for this project mostly lie in government regulation of pharmaceutical testing. The device is designed as an alternative to 2D and 3D cell culture methods, high-throughput screening, and the current gold standard, organs-on-chips. For these reasons, the FDA will have great interest in this device. As a result, a new standard operating procedure (SOP) will need to be established and monitored for our device. Globally, ISO will have to verify the quality of the device in order to maintain the current global standard for preclinical research. Additionally, one must consider that companies who have used our device to prevent monetary loss will generate more revenue. This can be used for lobbying political institutions and garnering support from officials.

6.8 Ethical Concerns

Our device proposes a new solution to an ethical problem that has plagued the scientific community for years. This ethical problem is clinical testing on animal subjects. Many people view this as cruel and inhumane. Our system offers a safe alternative to this problem. Instead of using animals for testing, human cells are used. This eliminates the need for animals and offers a direct view of a compounds effect on human cells.

A new ethical concern arises with the use of human cells. This new concern is with the use of organoids. The question may be posed, when would they be considered alive? They are live cells but when they start functioning as a tissue would they be considered alive? Some may argue that it is unethical to try and recreate organs which are naturally occurring in humans.

6.9 Health and Safety

Our device is intended to be used as a preclinical method for screening drugs before they enter clinical trials. In operation our device will alert researchers of any potential harmful effects on different organs. Our device also accounts for microvascular drug absorption and organ to

organ interactions. With an accurate model for drug screening, researchers can prevent harmful compounds from reaching clinical trials, thus protecting the health and safety of participants in these studies.

6.10 Manufacturability

The decellularization and cell seeding system was manufactured using the machine shop at Higgins Laboratory at Worcester Polytechnic Institute. All components used in our prototype were conventional materials that were processed with a laser cutter, drill press and tap. Common electrical components were used including a breadboard, peristaltic pump, pressure transducer, Arduino Uno, and fans. Acrylic cement and caulking were used to prevent any leakages of fluid at the acrylic interfaces. Upon completion of the SolidWorks drawings the manufacturability is simple. Because of its simplicity, there is potential, in later device iterations, for mass production.

VII. Discussion

Over the course of this project, many feasibility and validation studies have been conducted, results collected, and data analyzed. Each experiment was necessary to prove that the design was validated properly and fulfilled the client's needs. These experiments include the verification of decellularization, cell seeding and cell viability.

7.1 Decellularization

One of the primary function of this system is to facilitate the decellularization process of leaves. To test this, three leaves were attached to the system. The leaves used in this experiment had their cuticles removed prior to attachment to the system. The protocol for cuticle removal can be found in Appendix A: Protocol for Plant Decellularization. After attachment to the system the leaves were then exposed to a sequence of solutions as listed in the plant decellularization protocol. After the decellularization process, decellularization was visually confirmed by the leaves' clear translucent appearance.

A leaf was processed, embedded in paraffin wax, and cut into 8µm section using a microtome. Decellularization was histologically confirmed with a Safranin O and Fast Green stain. The Safranin O and Fast Green stain makes cellular material appear blue, while the cell walls appear pink. The results can be seen in Figure 28. Compared to the control image in Figure 28A and the example from the Gaudette lab in Figure 28B, the leaf from our system was successfully decellularized. This can be justified by its lack of blue coloration and intact cell wall structures.

7.2 Cell seeding

An important step of testing out design is ensuring cells can be seeded on decellularized leaves. To test cell attachment to the leaf, cells were first loaded with quantum dots. Quantum dots are ideal for this application because of their narrow spectrum of excitation. This narrow

spectrum reduces the auto-fluorescence of the surroundings. The quantum dot loaded cells were then seeded onto the leaf according to the cell seeding protocol described in Appendix B: Plant Co-culture Protocol. Cell seeding was found to be successful by the presence of quantum dots on the decellularized leaf.

7.3 Cell viability

The fact that the cells have adhered to the surface of the decellularized leaf does not insure that the cells are viable. The agents used to decellularize the leaf are toxic to the cells, and trace amounts of these agents could cause the cells to die. The viability of the cells is essential to conducting any further studies on the cells and a compounds effects. To assess cell viability, cells were seeded onto a decellularized leaf. Once seeded the cells were incubated for an additional 24 hours. The leaf was then stained according to the protocol in Appendix D: Protocol for live/dead stain. The results of this stain can be seen in Figure 30.

Ethidium homodimer-1 appears red and only stains the nuclei of dead cells. Phalloidin appears green and stains the F-actin in the cytoskeleton of all cells. Finally Hoechst stains all nuclei blue. As a result of the overlaid images, live cells will appear to have a green cytoskeleton with blue nuclei. Dead cells will appear to have green cytoskeletons with purple nuclei. In Figure 30 there are few cells with purple nuclei and several cells can be spotted with blue nuclei. These results prove that not only are the cells capable of adhering to the surface of the leaf, they can also remain viable. The results collected throughout these studies prove that decellularized leaves can act as a platform for tissue engineering and drug screening applications.

VIII. Conclusion and Recommendations

The team has designed and created a system for decellularization and cell seeding of plants that maintains sterility, cell viability and the structural integrity of the leaves. The design is a low-cost product which utilizes active vasculature and maintains cell viability. Using our design, the team was able to decellularize leaves using a combination of different detergents. Additionally, the team was able to seed cells onto the decellularized leaves and maintain cell viability for 24 hours. Overall, this system has met the client's expectations and has allowed for the progression of research involving the combination of plant material and animal cells for tissue engineering and drug screening applications.

Throughout the completion of this design process, future improvements and areas for continued research were highlighted and documented. Cell viability is essential to conducting further studies of a compound's effect. Although cell viability was determined using our system, other methods are also available in order to determine cell viability in a more quantitative method.

One possible way to test cell viability is through the use of alkaline phosphatase. Alkaline phosphatase is produced by specific cells, such as bone and liver cells, when they are healthy and thriving. When these specific cells are unhealthy, they do not produce the alkaline phosphatase. When present, alkaline phosphatase can be stained and imaged using light microscopy. This is important as some materials on the leaf produce auto-fluorescence when using fluorescence microscopy. The auto-fluorescence makes identifying structures on the leaf difficult. If alkaline phosphatase were to be imaged for, using light microscopy, the health of the cells can be assessed. Due to budget constraints, alkaline phosphatase cells were not able to be purchased for

research purposes. However, if this project were to be completed in the future with more funding, alkaline phosphatase cells could prove to be very useful in determining cell viability on a leaf.

The system was created in order to facilitate the growth of different cell types on each leaf. In the future, it is hoped that this system is able to facilitate the growth of different organoids, originating from different cell types on different leaves. By growing entire organoids on the same perfusion system, a biomimetic body could be created for drug screening purposes.

The system that was created was designed in such a way to allow for cell type to cell type communication between the different leaves. The tubing and reservoir aim to allow the cells to communicate through chemical signals passed through the media. If this project were to be advanced in the future, a methodology for collecting data on evidence of cell interactions and further organoid interactions should be established.

Overall, the system was created in order to overcome the current limitations of the preclinical drug screening processes such as organs-on-chips. It would be ideal to perform drug screening activities with our design to determine if this is possible. Drug screening activities may include introducing a drug to the system and then collecting data on the different cell type and organoid responses.

IX. References

[1] M. Herper, "The Truly Staggering Cost of Inventing New Drugs," in *Forbes*, ed, 2012.

[2] (2012). *New Drug Development and Review Process*. Available: http://www.fda.gov/drugs/developmentapprovalprocess/smallbusinessassistance/ucm053131.htm

[3] S. Bhatia and D. Ingber, "Microfluidic organs-on-chips," *Nature biotechnology*, pp. 760-772, 2014.

[4] J. Heemskerk, "High Throughput Drug Screening," *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*, vol. 5, pp. 19-21, 2004.

[5] J. W. Armstrong, "A Review of High-Throughput Screening Approached for Drug Discovery," HTS Consulting Ltd.1999.

[6] J. Giacomotto, and Ségalat, L., "High-throughput screening and small animal models, where are we?," *British Journal of Pharmacology*, vol. 160, pp. 204-216, 2010.

[7] H. A. Kenny, Madhu Lal-Nag, Erin A. White, Min Shen, Chun-Yi Chiang, Anirban K. Mitra, Yilin Zhang, Marion Curtis, Elizabeth M. Schryver, Sam Bettis, Ajit Jadhav, Matthew B. Boxer, Zhuyin Li, Marc Ferrer, and Ernst Lengyel., "Quantitative High Throughput Screening Using a Primary Human Three-dimensional Organotypic Culture Predicts in Vivo Efficacy," *Nature Communications*, vol. 6, 2015.

[8] J. T. DiPiro, W. J. Spruill, W. E. Wade, R. A. Blouin, and J. M. Preumer, "Lesson 1: Introduction to Pharmacokinetics and Pharmacodynamics," in *Concepts in Clinical: Pharmacokinetics*, 5 ed: American Society of Health-System Pharmacists, 2010, pp. 1-18.

[9] E. Novosel, C. Kleinhans, and P. Kluger, "Vascularization is the key challenge in tissue engineering," *Adv Drug Deliv Rev.*, vol. 63, pp. 300-11, 2011.

[10] C. Parng, W. Seng, C. Semino, and P. McGrath, "Zebrafish: a preclinical model for drug screening.," *Assay Drug Delivery Technology*, vol. 1, pp. 41-8, 2002.

[11] G. M. Cooper and R. E. Hausman, "Cell Walls, the Extracellular Matrix, and Cell Interactions," in *The Cell: A Molecular Approach*, 5th ed Washington, D.C.: ASM, 2009, pp. 571-599.

[12] W. V. Dashek and M. Harrison, *Plant Cell Biology*. Enfield, NH: Science, 2006.

[13] *A Closer Look at Plant Classification, Parts, and Uses*, 1st ed. New York: Britannica Education, 2012.

[14] *Xylem*. Available: http://www.botany.uwc.ac.za/SCI_ED/grade10/plant_tissues/xylem.htm

[15] (2009). *Reversion from Xylem Vessels to Tracheids*. Available: http://www.mapoflife.org/topics/topic_451_reversion-from-xylem-vessels-to-tracheids/

[16] "Phloem," ed. Plant Tissues (Std 8): University of the Western Cape: Department of Biodiversity and Conservation Biology.

[17] *A Closer Look at Plant Reproduction, Growth, and Ecology*, 1st ed. New York: Britannica Educational, 2012.

[18] T. W. Gilbert, T. L. Sellaro, and S. F. Badylak, "Decellularization of tissues and organs," *Science Direct*, vol. 27, pp. 3675-3683, 2006.

[19] P. Crapo, T. Gilber, and S. Badylak, "An overview of tissue and whole organ decellularization processes.," *Biomaterials*, vol. 32, pp. 3233-43, 2011.

[20] C. L. Dym, P. Little, and E. J. Orwin, *Engineering Design: A Project-based Introduction*. New York: John Wiley, 2014.

[21] R. Bear and D. Rintoul, 2013

[22] ISO/IEC, (2003). ISO 13485:2003, Quality management systems — Requirements for regulatory process. Geneva, Switzerland: ISO/IEC.

[23] Richter, S. G. (n.d.). Sterility Testing -Essential Things you must know. Retrieved April 13, 2016, from http://www.microtestlabs.com/documents/wp_sterility-testing.pdf

[24] ISO/IEC, (2004). ISO 129-1:2004, technical drawings – Indication of dimensions and tolerances – Part 1: General Principals. Geneva, Switzerland: ISO/IEC.

[25] Equipment Authorization. (2015, October 21). Retrieved April 13, 2016, from https://www.fcc.gov/engineering-technology/laboratory-division/general/equipment-authorization

[26] Cannon, L. W., Whittington, N. O., & Elliott, R. A. (n.d.). Recommended C Style and Coding Standards. Retrieved April 13, 2016, from http://ieng9.ucsd.edu/~cs30x/indhill-cstyle.html

[27] "Biopharmaceutical Research and Development: The Process Behind New Medicines", *Pharma*, 2016. [Online]. Available: http://www.phrma.org/sites/default/files/pdf/rd_brochure_022307.pdf. [Accessed: 15- Apr- 2016].

[28] "Physiology of Circulations", *Cardiovascular & Exocrine System*, 2013. [Online]. Available: https://anatomygroup.wordpress.com/cardiovascular-system/blood-vessel/physiology-of-circulations/. [Accessed: 23- Apr- 2016].

[29] J. Brselton, "Structure of Plants", *Botany Readings*, 2016.

[30] "Pearson - The Biology Place", Phschool.com, 2016. [Online]. Available: http://www.phschool.com/science/biology_place/biocoach/plants/walls.html. [Accessed: 25- Apr- 2016].

Appendix A: Protocol for Plant Decellularization

This is the protocol used for plant decellularization provided by the Gaudette Laboratory.

Protocol for Plant Decellularization

Used for spinach (and other) leaves, parsley stems

Materials:

- Cannulas (20G)
- Hexanes
- PBS 1x
- DI H2O
- 10X SDS solution in DI H2O
- 0.1% Triton-X with 10% bleach (Cl tablets) in DI H2O
    - Ex: If using 4g Cl tablets, place in 4L of solution.
- Same protocol applies if using Gaudette Lab decellularization apparatus or custom set-up.
    - Gaudette decellularization apparatus requires ~2-4L of each solution to function efficiently. Custom decellularization apparatus should use an appropriate amount of solution depending on size.

Protocol:

1. Wash leaves and/or stems in DI water.
2. Submerge in hexanes and wash vigorously for 2 minutes. Remove and rinse in 1x PBS for 2 minutes **(Repeat 3x times)**.
3. Cannulate leafs and parsley stems via the stem, affixing cannulas with glue or suture. If using glue, use sparingly to ensure flow is not obstructed.
4. Once leaves are cannulated, allow glue to dry (if used), keeping plant material hydrated with DI H2O.
5. Use syringe to validate flow: perfuse a small amount (~1mL) of DI water through the cannula to verify that there is unobstructed flow through the leaf.
6. Affix plant materials to decellularization set up.
7. Attach 10x SDS solution to the set up and begin flow. Monitor plant materials to ensure proper flow through the leaf vasculature, modifying flow rate to ensure a slow, steady drip.
    a. Rapid flow rate will deplete SDS too quickly, whereas too little flow runs the risk of dehydrating the plant material and damaging the plant structure.
8. Maintain in SDS for 5 to 7 days in order to decellularize plant material, until leaves and stems become more transparent in appearance. Green coloration at this step is normal and not indicative of an unsuccessful decellularization.
9. Remove 10x SDS and attach Triton-X/Bleach solution to set up.

10. Maintain set up in Triton-X/Bleach for 24-48 hours, or until leaves and stems have become clear/transparent. This solution should purge any remaining coloration from the plant matter.
11. Remove Triton-X/Bleach solution and attach DI H2O to set up. Perfuse leaves/stems in DI H2O for 24 hours.
12. Remove leaves and stems, store in DI H2O at 4 degrees Celsius until needed.

Appendix B: Plant Co-culture Protocol

Plant Co-culture (For PLANTIMAL MQP):

1. Decellularize leaf
2. Sterilization:
    - In 10cm dish containing decelled leaf, add the following:
        - 10mL 10% bleach
        - 1mL 0.1% Triton-X
    - Agitate gently on shaker table for 30 minutes
    - Transfer leaf to Laminar Flow hood
    - Aspirate sterilization solution
    - Rinse 3x with sterile water
    - Rinse 3x with sterile PBS
    - Section leaf for plating with sterile tweezers and scalpel/razor
    - NOTE: Leaf should change from opaque (after the decell process), to translucent (after sterilization)
3. Incubate the leaf in media overnight to equilibrate leaf to salinity of media
4. Chelate leaf with sterile 2mM EDTA in PBS
    i. Add EDTA to fresh well
    ii. Introduce leaf to EDTA well and gently rock back and forth by hand for 45-60 seconds taking care to coat whole leaf section
    iii. GENTLY dry leaf section on edge of Kimwipe to remove excess EDTA
5. Secure leaf solidly to the bottom of a petri dish
    a. Have been using Gaudette-Pins Transwell but a sterile washer should suffice
    b. Want to make sure surface is taut and solid to stiff bottom of petri dish
6. Seed cells to surface

Appendix C: Protocol for histological stain for cell wall and cytoplasm

Brief protocol for fixation and staining of both normal/ decellularized leaf samples:

1. Fix on “routine” cycle in tissue processor
2. Section at 14 um
3. Deparaffinize, bring to H20 (per usual method)
4. Stain 1 hour, aqueous Safranin O (1% w/v)
5. Wash in DI until sections clear of residual dye (5 mins, approx)
6. Dehydrate up to 95% EtOH (2-5 mins/ step)
7. Dip 10s in 95% EtOH + Fast Green FCF (0.1% w/v)
8. Wash (2x – 2 min each step) in 100% EtOH
9. Clear in at least 2 changes of xylene, mount, image

Expanded protocol for Safranin O/Fast Green (Sass’s Method)

1. Make up safranin O (1% w/v in DI water)
2. Make Fast Green (0.1% w/v in 95% EtOH)
3. Deparaffinize and hydrate in two changes of xylene; two changes 100% EtOH; 1 change 95% EtOH, 1 change 70% EtOH; 1 change DI H20 (2 mins, each step).
4. Stain for 1 hour in aqueous Safranin O.
5. Rinse in tap water until sections cleared of residual dye (~ 5 mins)
6. Dehydrate to 95% EtOH (2 mins).
7. Dip for 10 seconds in Fast Green stain.
8. Immediately wash in 100% EtOH (2x, 2 mins).
9. Clear in xylenes, mount with coverslip.

Appendix D: Protocol for live/dead stain

Solutions:

Solution 1:

- 1.0 mL Serum Free DMEM
- 2.0 µL Ethidium Homodimer-1

Solution 2:

- 1.0 mL Serum Free DMEM
- 2.0 µL Ethidium Homodimer-1

Phalloidin (AF 488 Phalloidin A12379, Invitrogen):

- 2.5% V/V Phalloidin in PBS
- 50 µL in 1950 µL PBS

Hoechst:

- 0.0167% Hoechst dye in PBS
- 0.5 µL in 3000 µL PBS

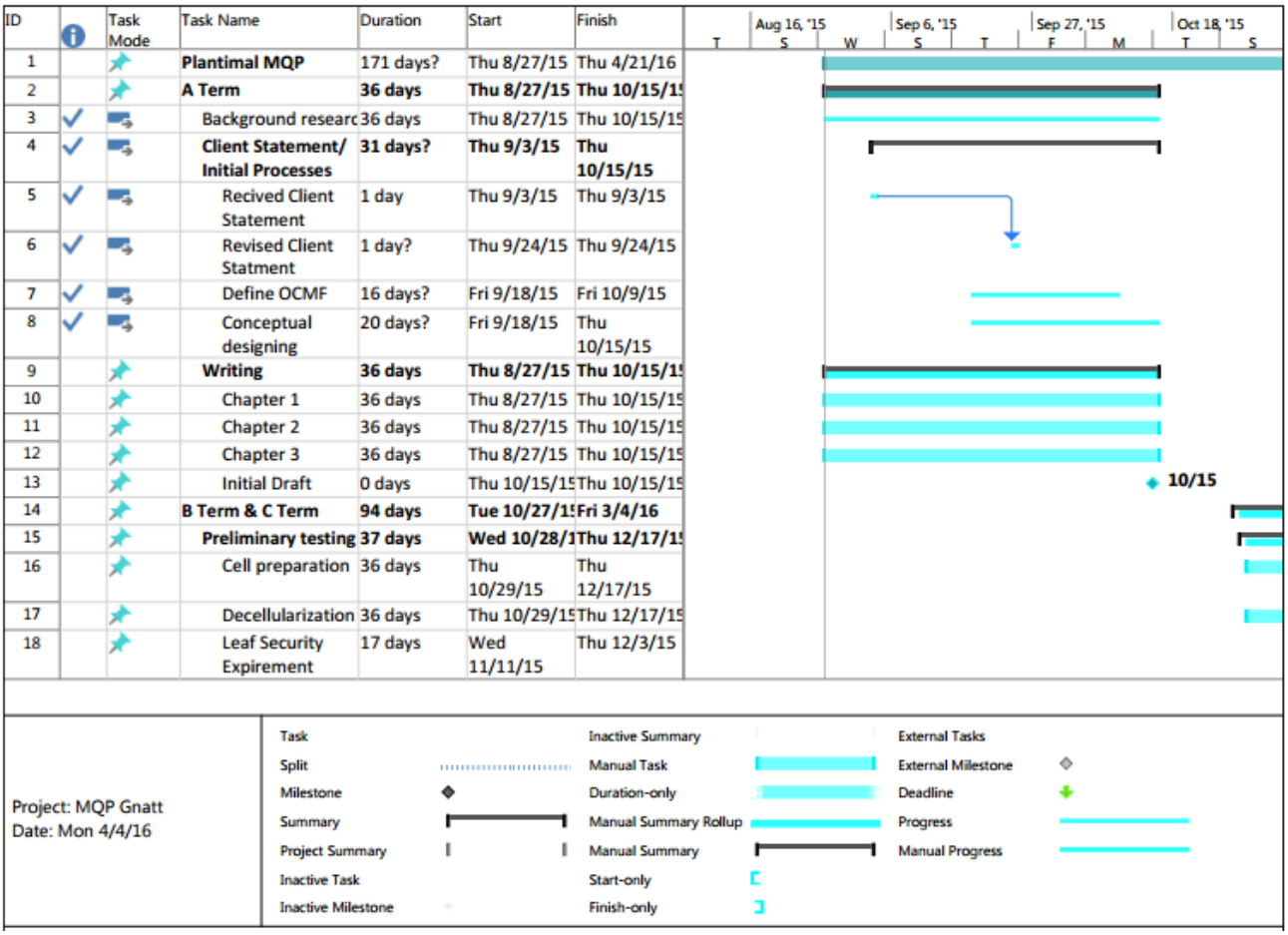
Process:

1. Turn off the lights
2. Mix solution 1 within 1 hour of use
3. Let cells sit with solution 1 for 15 minutes, then aspirate
4. Mix solution 2 within 1 hour of use
5. Let cells sit with solution 2 for 15 minutes, then aspirate
6. Wash cells with 1x PBS, 6 times
7. Fix cells with 4% phosphate buffered formaldehyde for 10 minutes
8. Rinse with PBS, 2 times
9. Add Triton-X solution, let sit 10 minutes, then aspirate
10. Rinse with PBS, 2 times
11. Block with BSA solution for 30 minutes
12. Add Phalloidin solution, let sit for 30 minutes, then aspirate
13. Rinse with PBS, 2 times
14. Add Hoechst solution, let sit for 3 minutes, then aspirate
15. Rinse with PBS, 2 times
16. Store at –20 degrees C

Appendix E: Gantt Chart

The Gantt Chart for this project was created using Microsoft Project Software. The resulting Gantt Chart can be seen below.

Zoomed in sections of the Gantt Chart are provided below for clarity.


ID
Task Mode
Task Name
Duration
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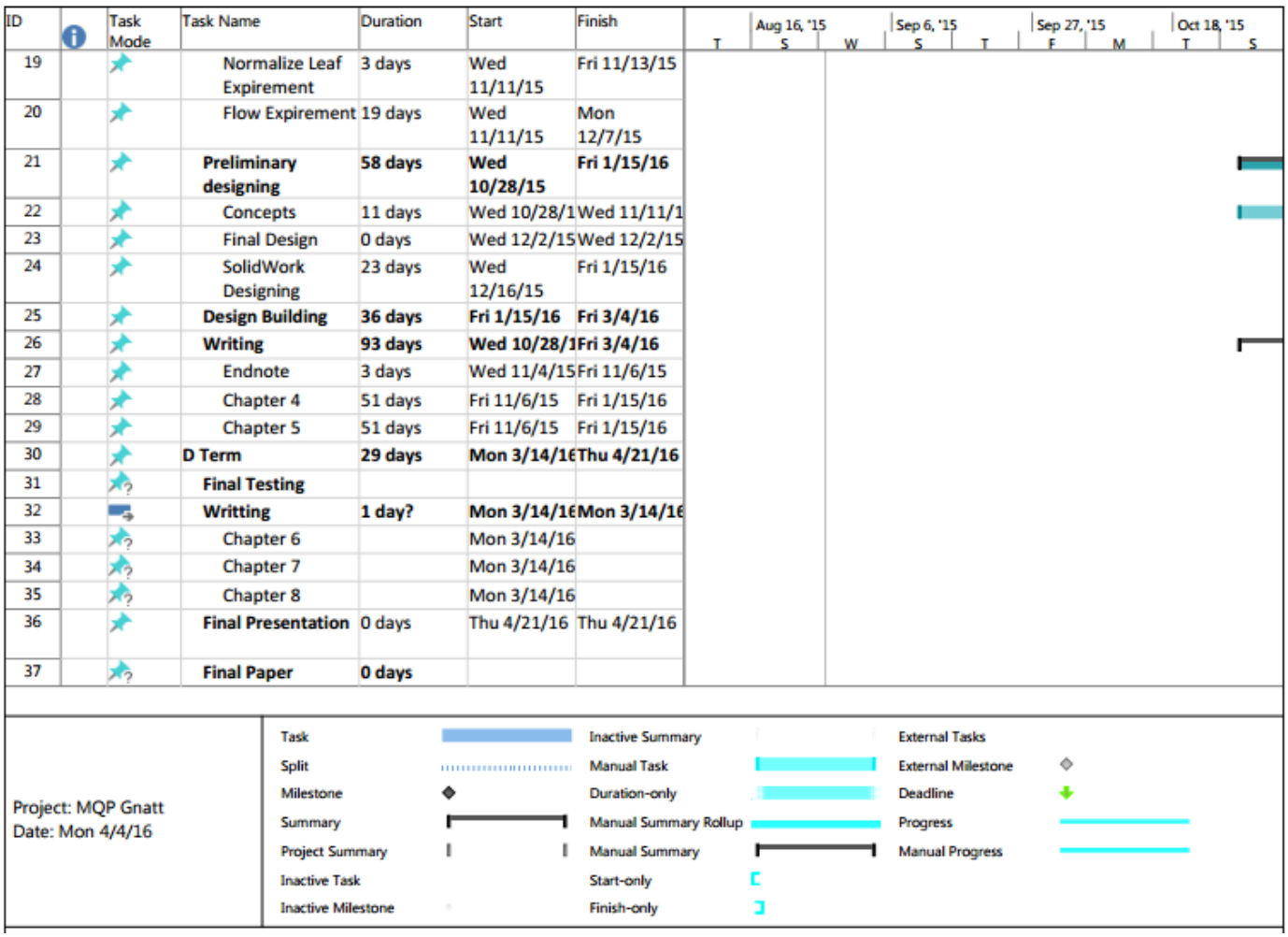
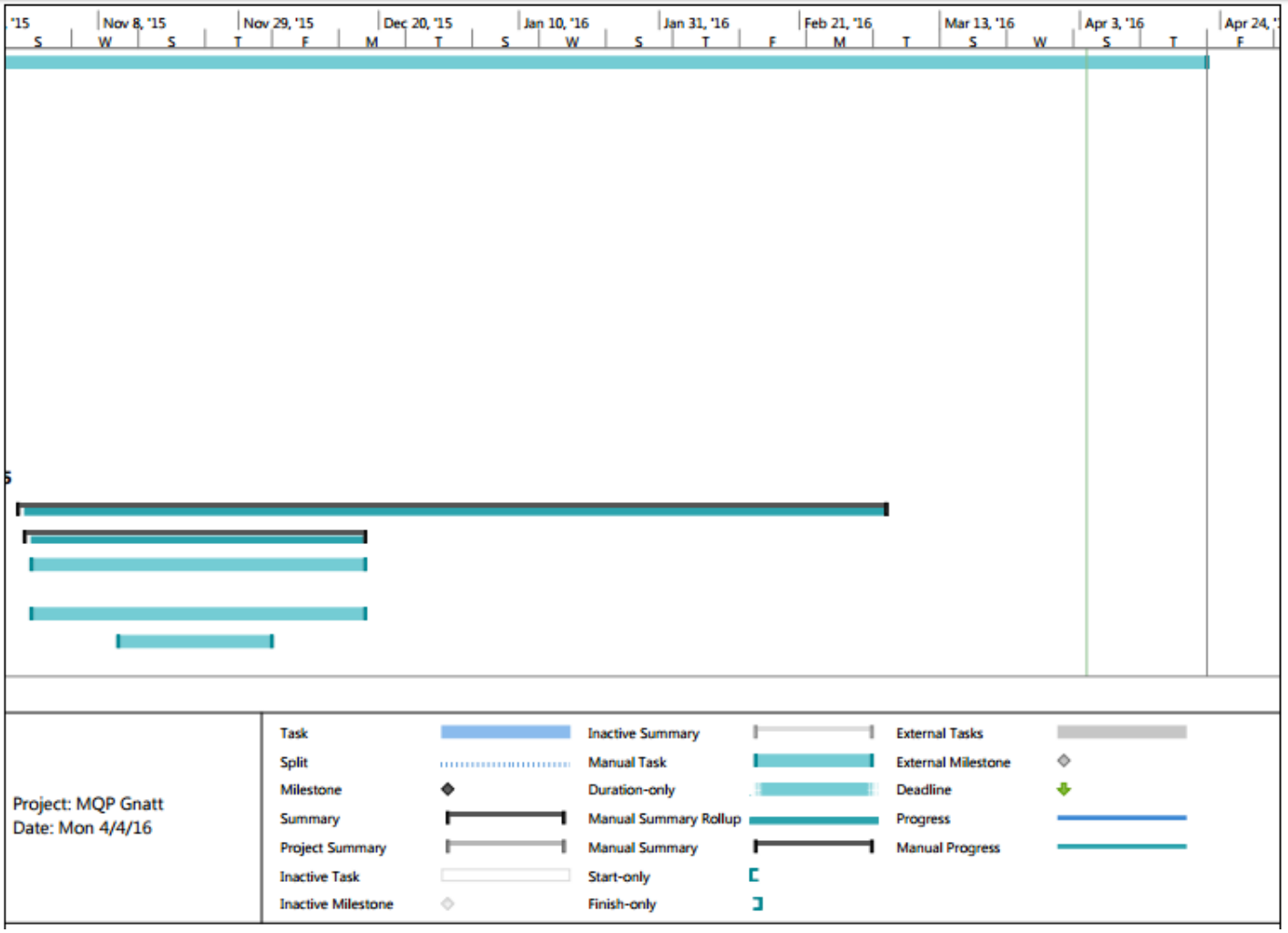
2 A Term 36 days Thu 8/27/15 Thu 10/15/15
3 Background researc 36 days Thu 8/27/15 Thu 10/15/15
4 Client Statement/ Initial Processes 31 days? Thu 9/3/15 Thu 10/15/15
5 Recived Client Statement 1 day Thu 9/3/15 Thu 9/3/15
6 Revised Client Statment 1 day? Thu 9/24/15 Thu 9/24/15
7 Define OCMF 16 days? Fri 9/18/15 Fri 10/9/15
8 Conceptual designing 20 days? Fri 9/18/15 Thu 10/15/15
9 Writing 36 days Thu 8/27/15 Thu 10/15/15
10 Chapter 1 36 days Thu 8/27/15 Thu 10/15/15
11 Chapter 2 36 days Thu 8/27/15 Thu 10/15/15
12 Chapter 3 36 days Thu 8/27/15 Thu 10/15/15
13 Initial Draft 0 days Thu 10/15/15 Thu 10/15/15
14 B Term & C Term 94 days Tue 10/27/15 Fri 3/4/16
15 Preliminary testing 37 days Wed 10/28/1 Thu 12/17/15
16 Cell preparation 36 days Thu 10/29/15 Thu 12/17/15
17 Decellularization 36 days Thu 10/29/15 Thu 12/17/15
18 Leaf Security Expirement 17 days Wed 11/11/15 Thu 12/3/15
Aug 16, '15
Sep 6, '15
Sep 27, '15
Oct 18, '15
10/15
Project: MQP Gnatt
Date: Mon 4/4/16
Task
Split
Milestone
Summary
Project Summary
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
External Tasks
External Milestone
Deadline
Progress
Manual Progress

ID	Task Mode	Task Name	Duration	Start	Finish
19		Normalize Leaf Expirement	3 days	Wed 11/11/15	Fri 11/13/15
20		Flow Expirement	19 days	Wed 11/11/15	Mon 12/7/15
21		**Preliminary designing**	**58 days**	**Wed 10/28/15**	**Fri 1/15/16**
22		Concepts	11 days	Wed 10/28/1	Wed 11/11/1
23		Final Design	0 days	Wed 12/2/15	Wed 12/2/15
24		SolidWork Designing	23 days	Wed 12/16/15	Fri 1/15/16
25		**Design Building**	**36 days**	**Fri 1/15/16**	**Fri 3/4/16**
26		**Writing**	**93 days**	**Wed 10/28/1**	**Fri 3/4/16**
27		Endnote	3 days	Wed 11/4/15	Fri 11/6/15
28		Chapter 4	51 days	Fri 11/6/15	Fri 1/15/16
29		Chapter 5	51 days	Fri 11/6/15	Fri 1/15/16
30		**D Term**	**29 days**	**Mon 3/14/16**	**Thu 4/21/16**
31		**Final Testing**			
32		**Writting**	**1 day?**	**Mon 3/14/16**	**Mon 3/14/16**
33		Chapter 6		Mon 3/14/16	
34		Chapter 7		Mon 3/14/16	
35		Chapter 8		Mon 3/14/16	
36		**Final Presentation**	0 days	Thu 4/21/16	Thu 4/21/16
37		**Final Paper**	**0 days**		

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Mar 13, '16
Apr 3, '16
Apr 24, '
S W S T F M T S W S T F M T S W S T F
Project: MQP Gnatt
Date: Mon 4/4/16
Task
Split
Milestone
Summary
Project Summary
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
External Tasks
External Milestone
Deadline
Progress
Manual Progress


'15
Nov 8, '15
Nov 29, '15
Dec 20, '15
Jan 10, '16
Jan 31, '16
Feb 21, '16
Mar 13, '16
Apr 3, '16
Apr 24, '
12/2
4/21
3/14
Project: MQP Gnatt
Date: Mon 4/4/16
Task
Split
Milestone
Summary
Project Summary
Inactive Task
Inactive Milestone
Inactive Summary
Manual Task
Duration-only
Manual Summary Rollup
Manual Summary
Start-only
Finish-only
External Tasks
External Milestone
Deadline
Progress
Manual Progress

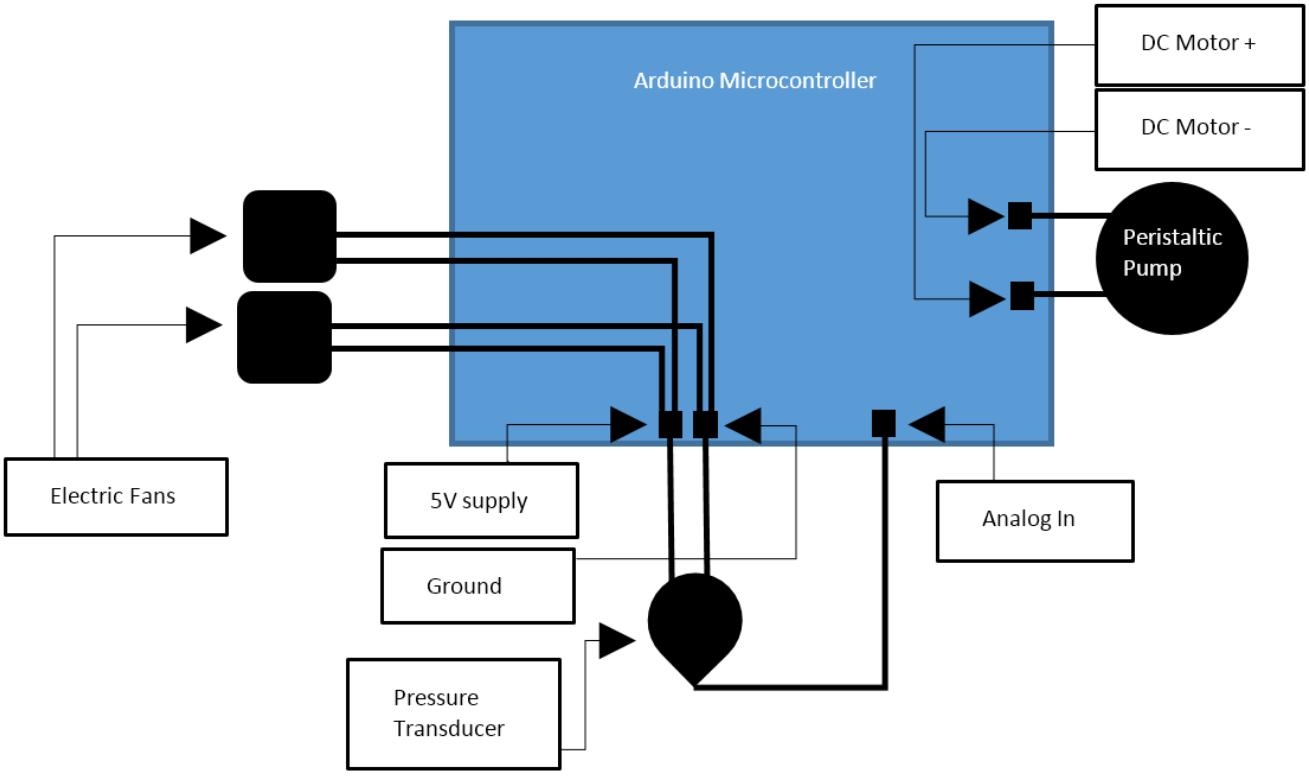
Appendix F: Software Code for Pump

This code was generated using the sample code from adafruit.com

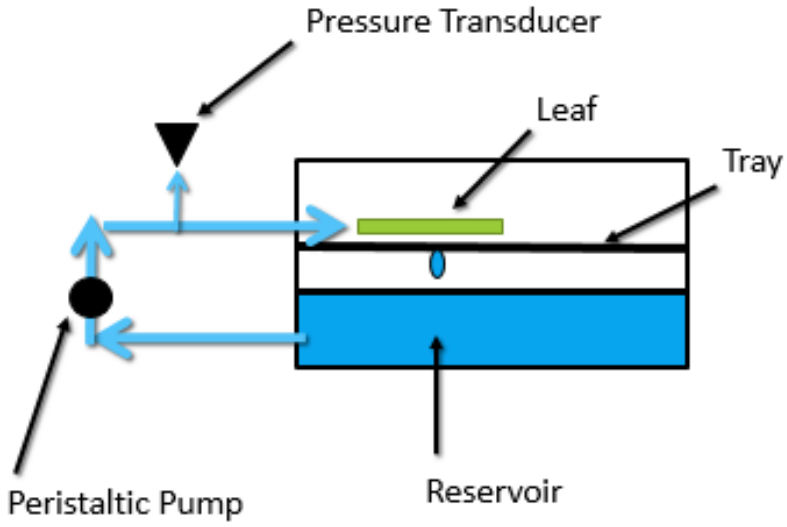
```
For use with the Adafruit Motor Shield v2
#include <Wire.h>
#include <Adafruit_MotorShield.h>
#include "utility/Adafruit_MS_PWMServoDriver.h"


// Create the motor shield object with the default I2C address
Adafruit_MotorShield AFMS = Adafruit_MotorShield();
// Or, create it with a different I2C address (say for stacking)
// Adafruit_MotorShield AFMS = Adafruit_MotorShield(0x61);


// Select which 'port' M1, M2, M3 or M4. In this case, M1
Adafruit_DCMotor *myMotor = AFMS.getMotor(1);
// You can also make another motor on port M2
//Adafruit_DCMotor *myOtherMotor = AFMS.getMotor(2);

void setup() {

  AFMS.begin();  // create with the default frequency 1.6KHz
  //AFMS.begin(1000);  // OR with a different frequency, say 1KHz

  // Set the speed to start, from 0 (off) to 255 (max speed)

  myMotor->setSpeed(150);
  myMotor->run(FORWARD);
  // turn on motor
  myMotor->run(RELEASE);
   Serial.begin(9600);
}
int i=150;
void loop() {
  // put your main code here, to run repeatedly:

 int Vs=5.1;
int sensorValue= analogRead(A0);
float Vout = sensorValue * (5.0 / 1023.0);
float p=(0.04/0.004)+(Vout/(Vs*0.004));
float Pf=p/0.13332239;
```

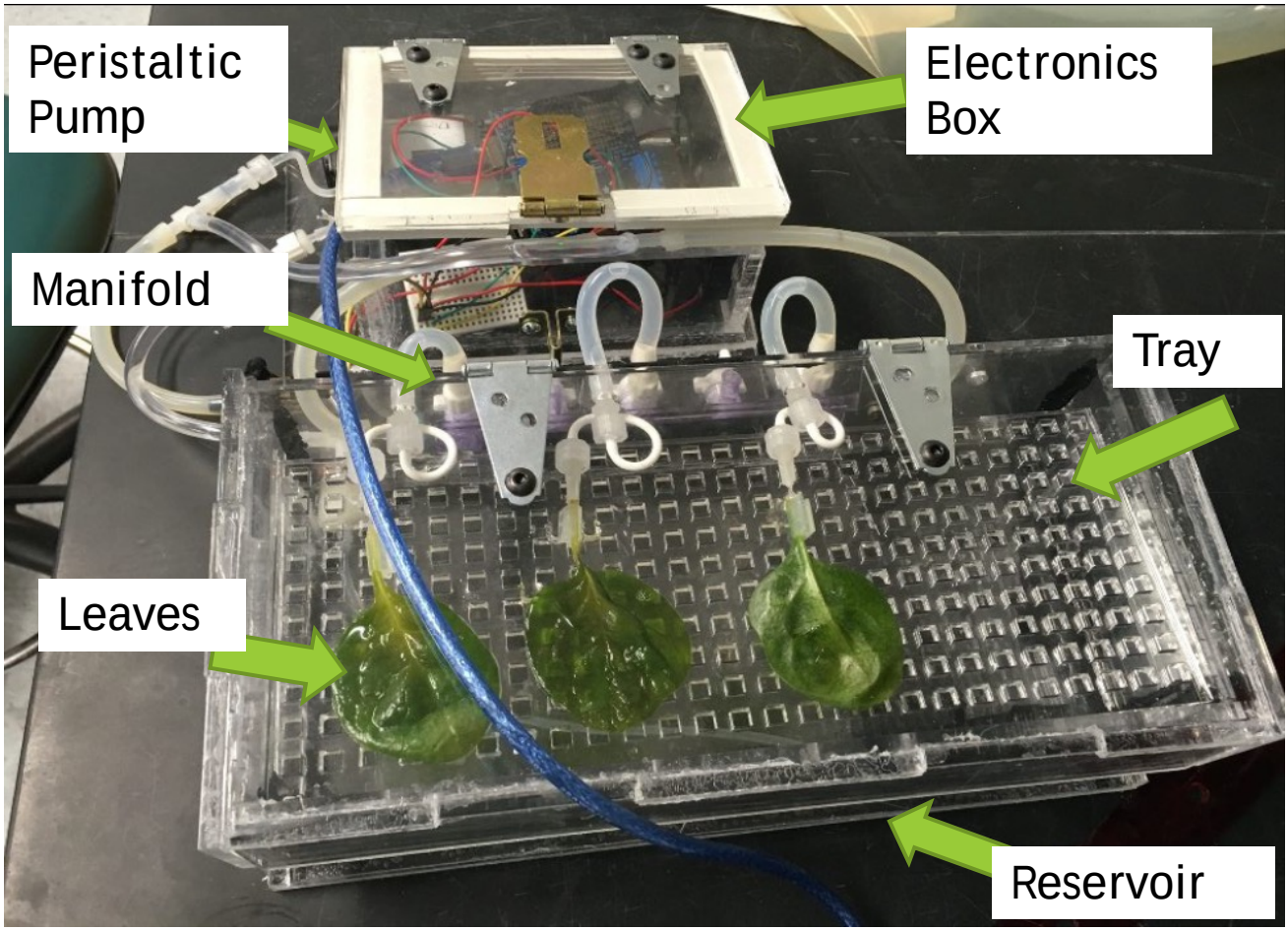
```
  if (Pf<=800) {
     i=i+5;
     myMotor->setSpeed(i);
 myMotor->run(FORWARD);

 Serial.print(Pf);
 Serial.print(" ");
 Serial.println(i);
 delay (500);
  }
  else

    { i=i-5;
      myMotor->setSpeed(i);
 myMotor->run(FORWARD);


  Serial.print(Pf);
 Serial.print(" ");
 Serial.println(i);
  delay(500);
 }
 if (i<=180)
 {i=180;}
 if (i>=255)
 {i=200;}
}
```

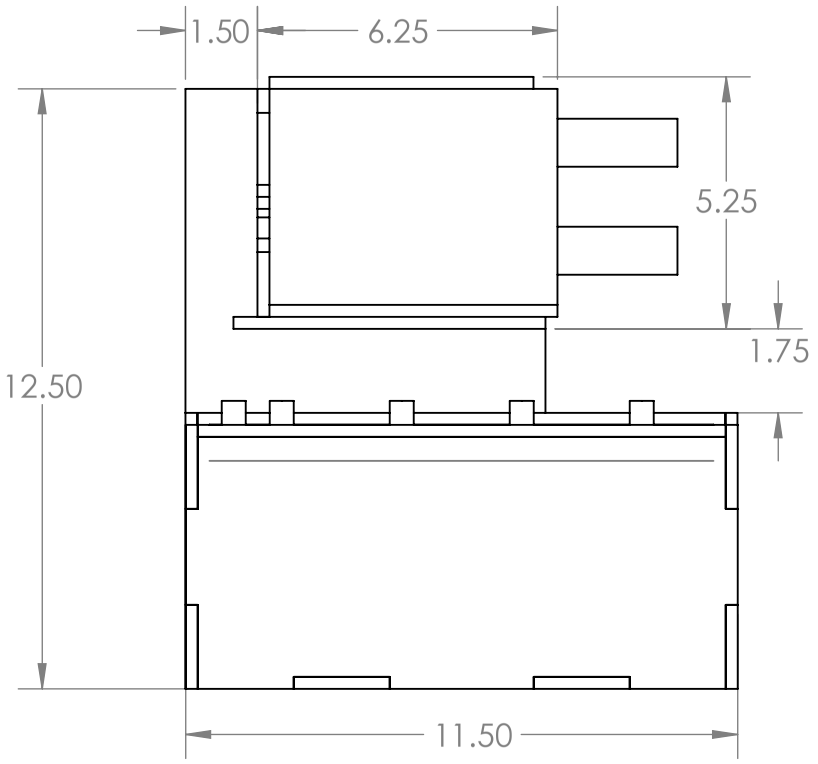
Appendix G: Circuit Diagram



Appendix H: Final Design and Solidworks Drawings







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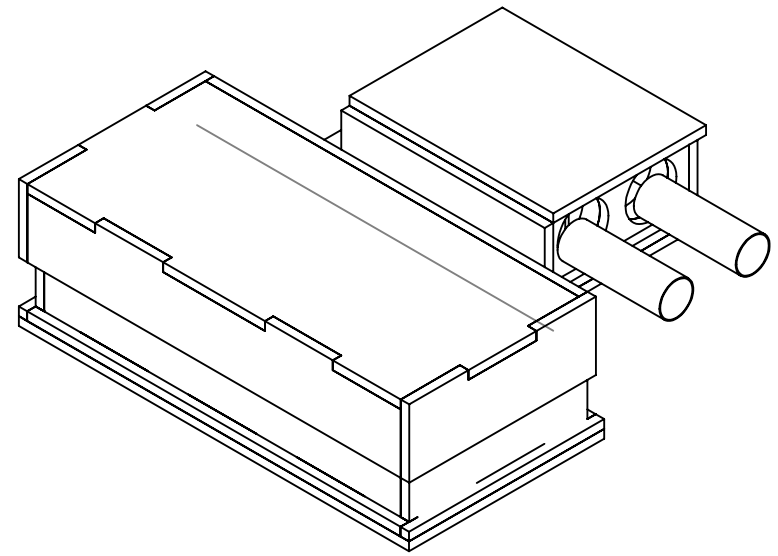

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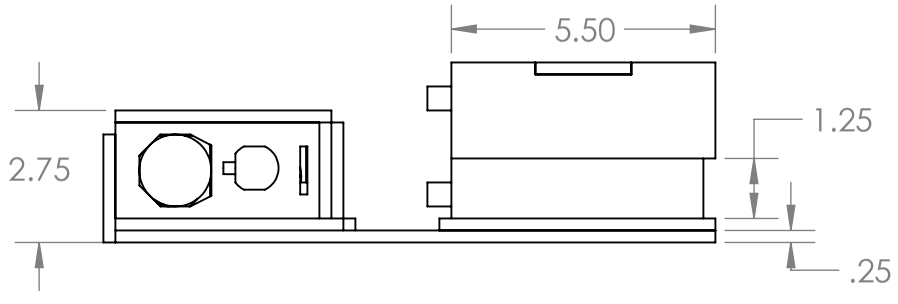

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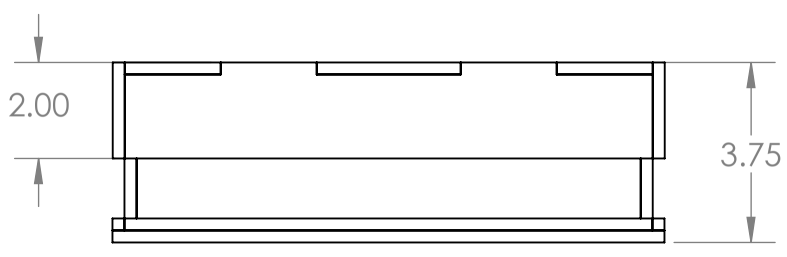

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APPLICATION		

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DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

DO NOT SCALE DRAWING

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CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

COMMENTS:

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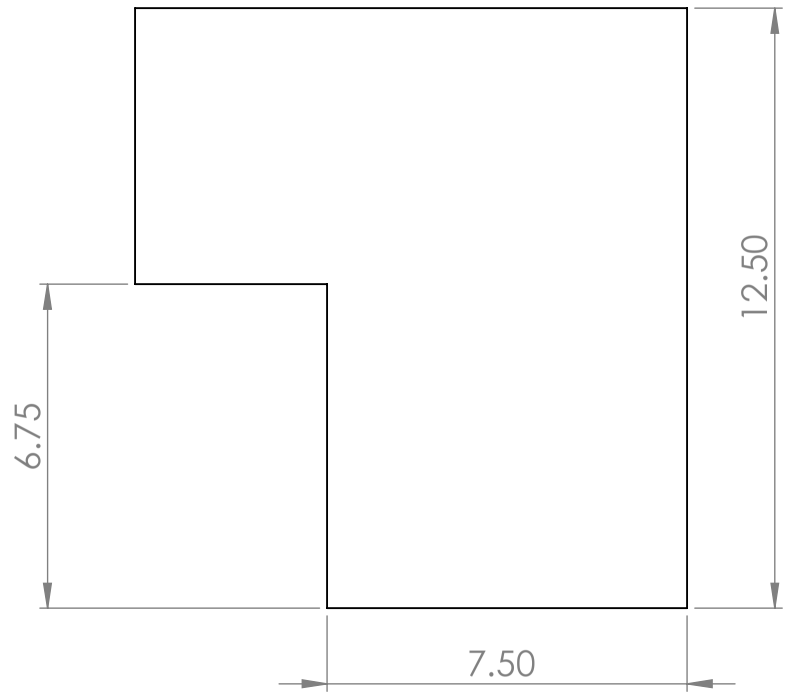

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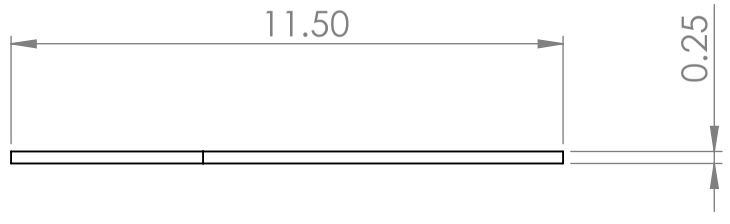

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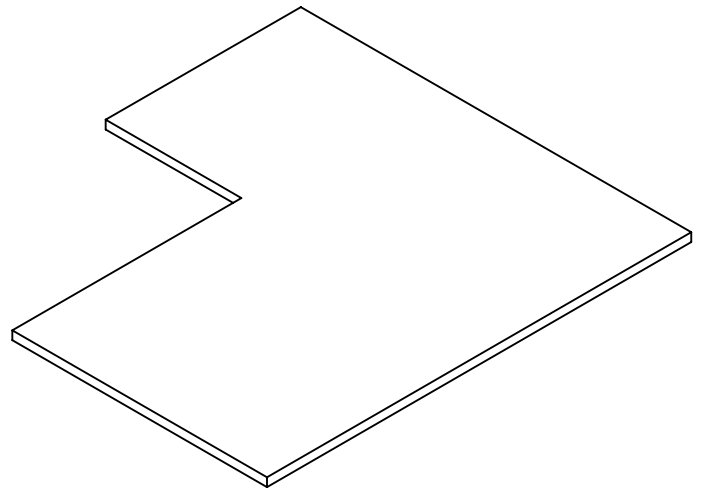

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MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

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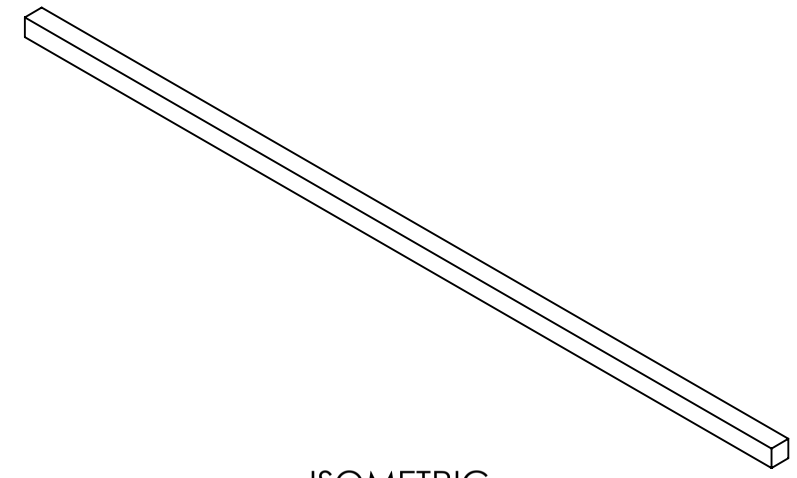

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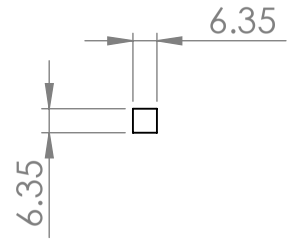

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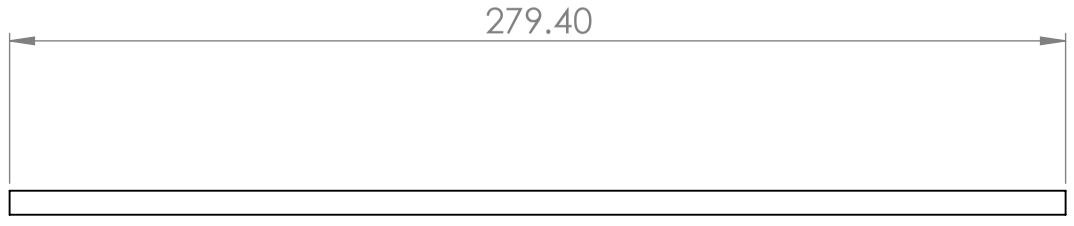

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CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

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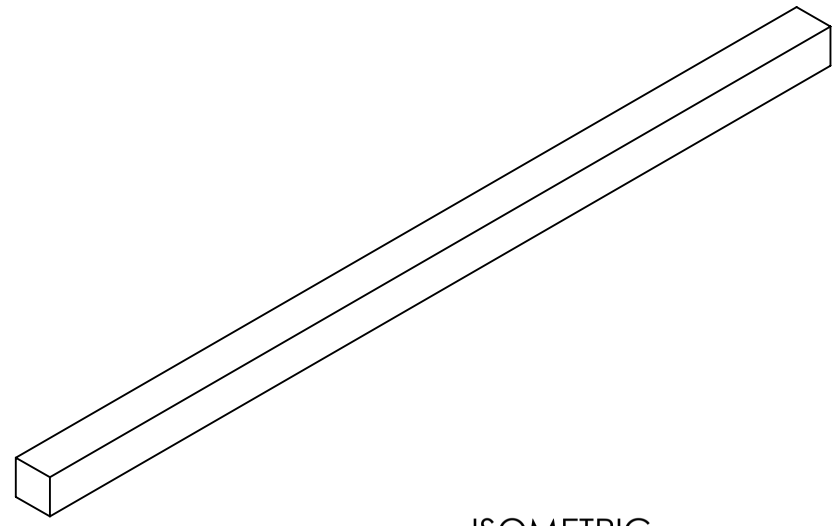

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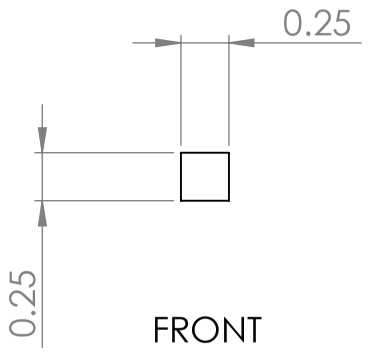

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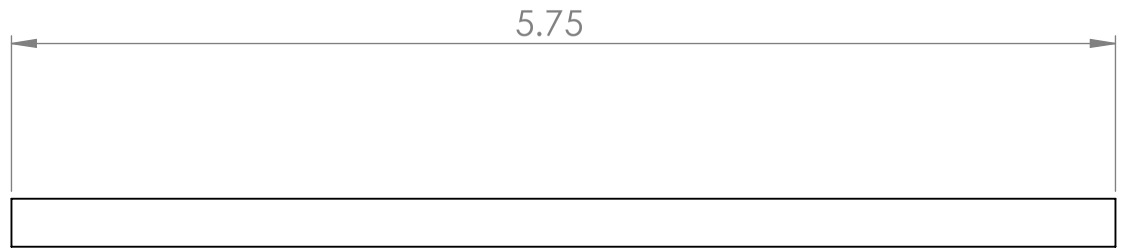

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FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

DO NOT SCALE DRAWING

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Q.A.	LG	

COMMENTS:

MQP ORGANS ON A VINE

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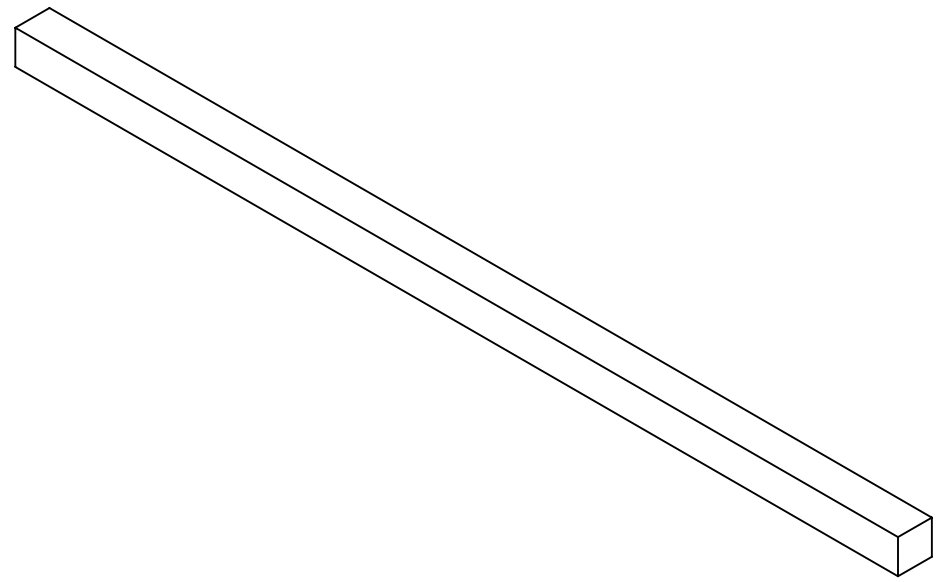

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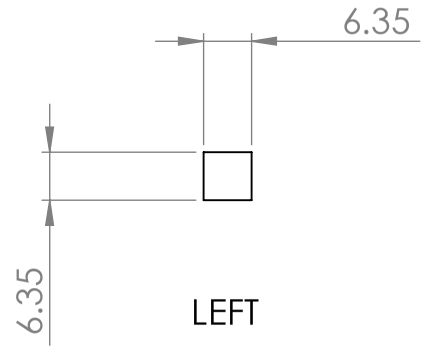

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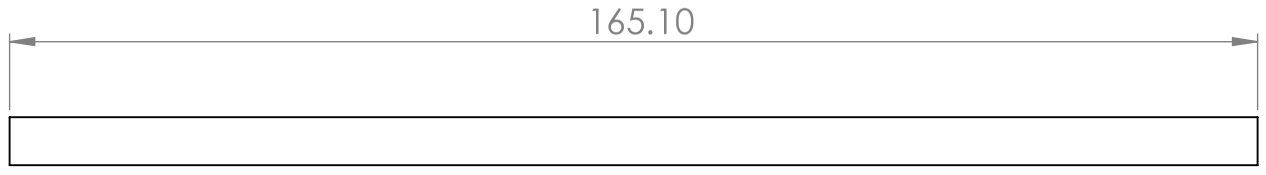

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ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

DO NOT SCALE DRAWING

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CHECKED	JDJ	4/8/2014
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MFG APPR.	RT	
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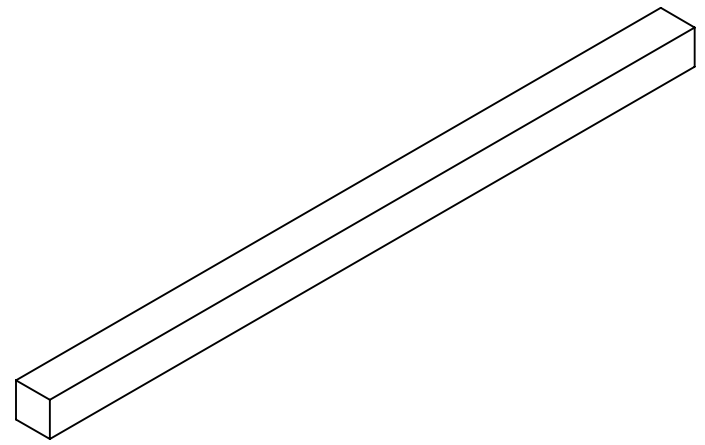

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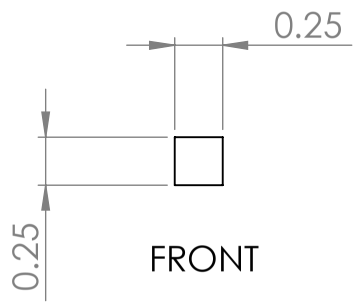

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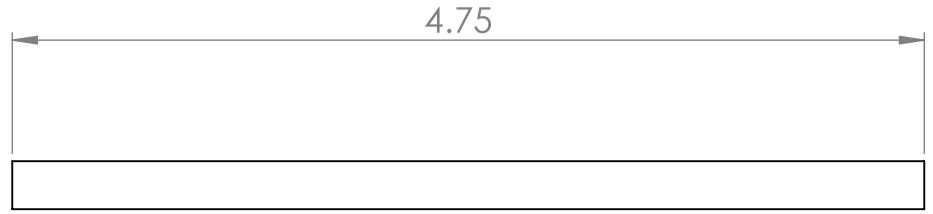

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APPLICATION		

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TOLERANCES:
FRACTIONAL ± 0.05
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TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

DO NOT SCALE DRAWING

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CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

COMMENTS:

MQP ORGANS ON A VINE

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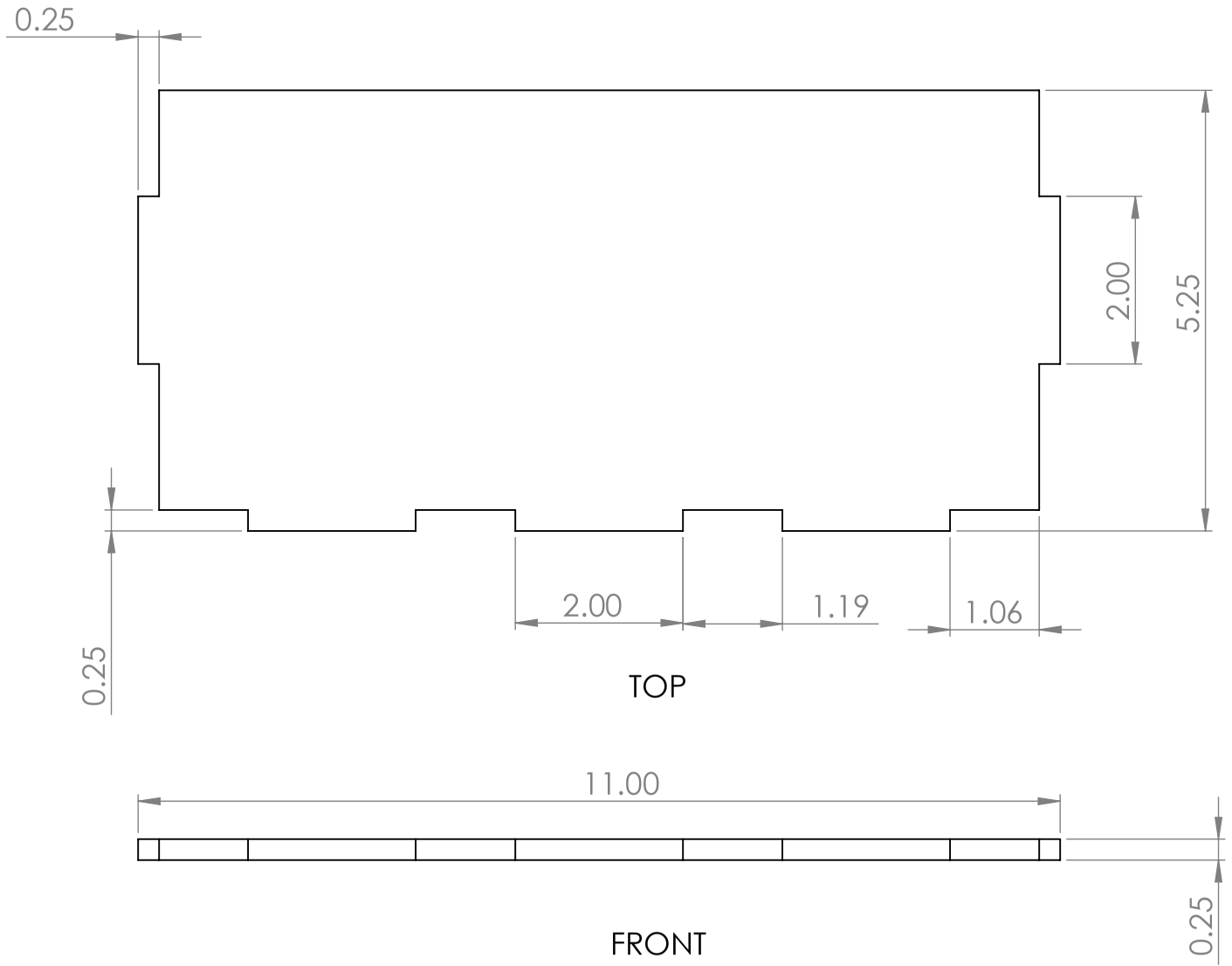

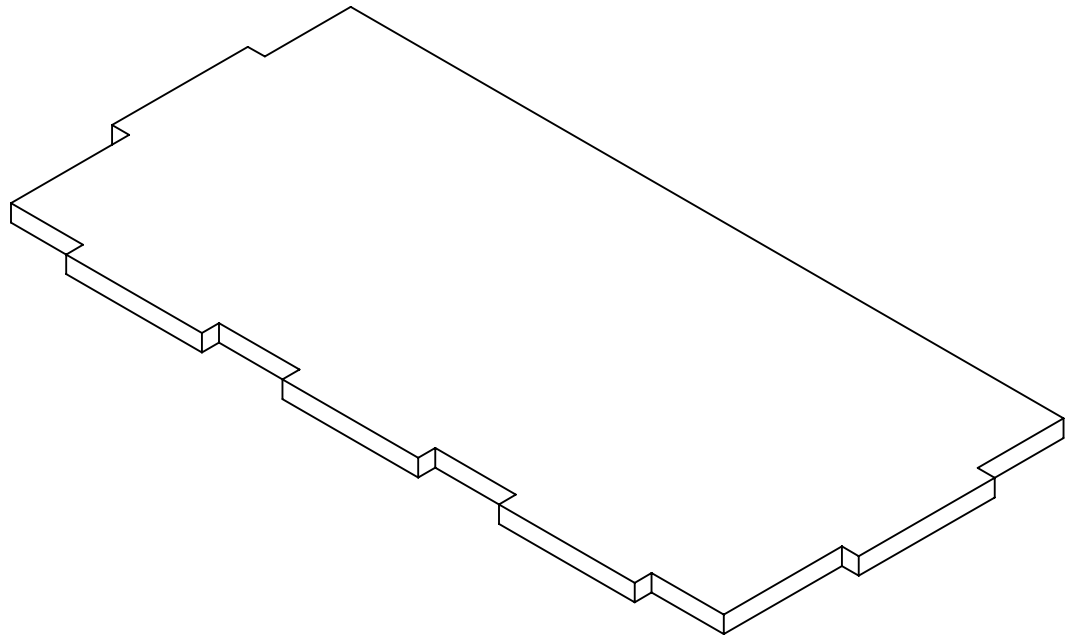

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CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

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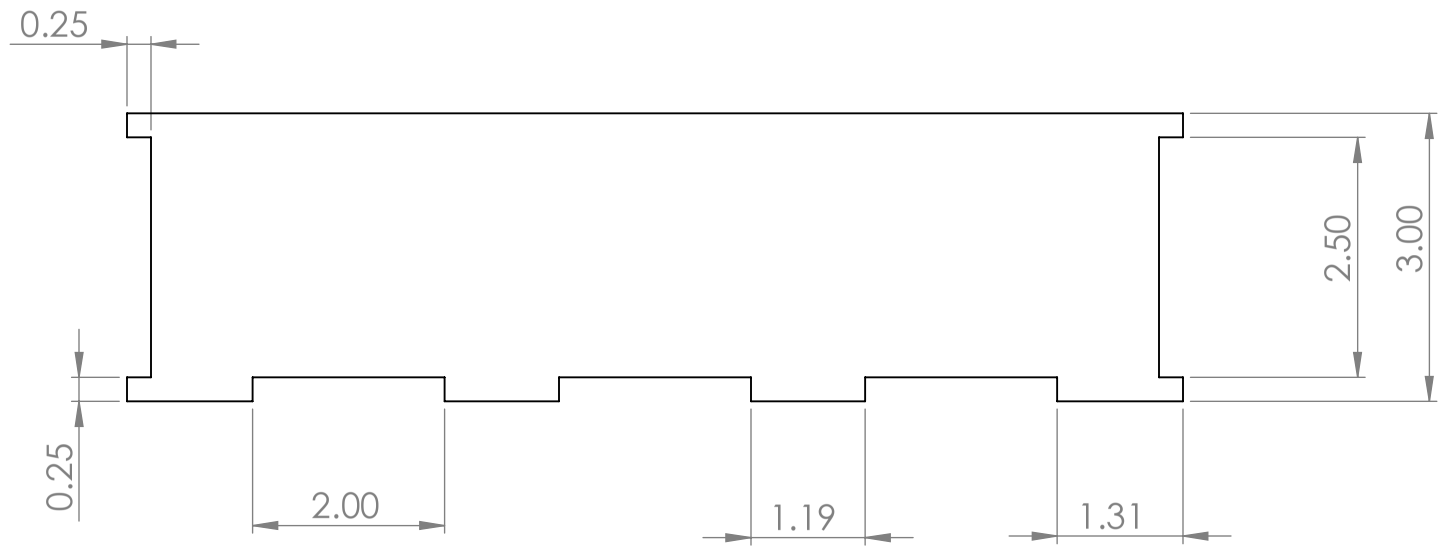

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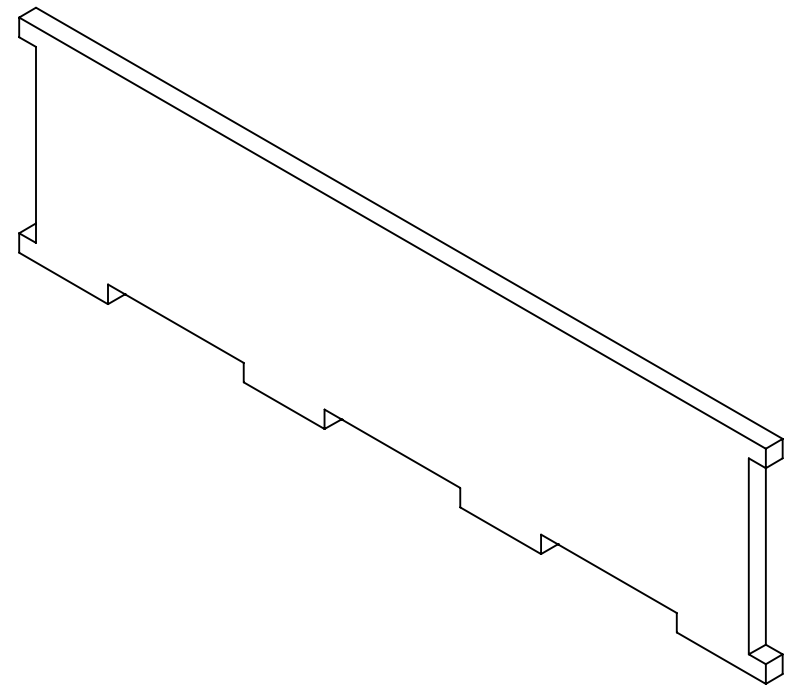

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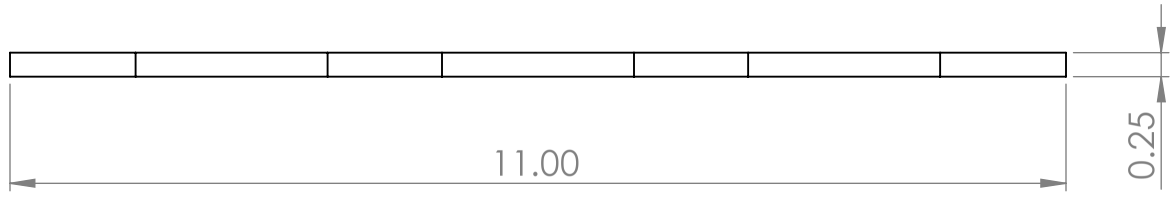

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NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
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Q.A.	LG	
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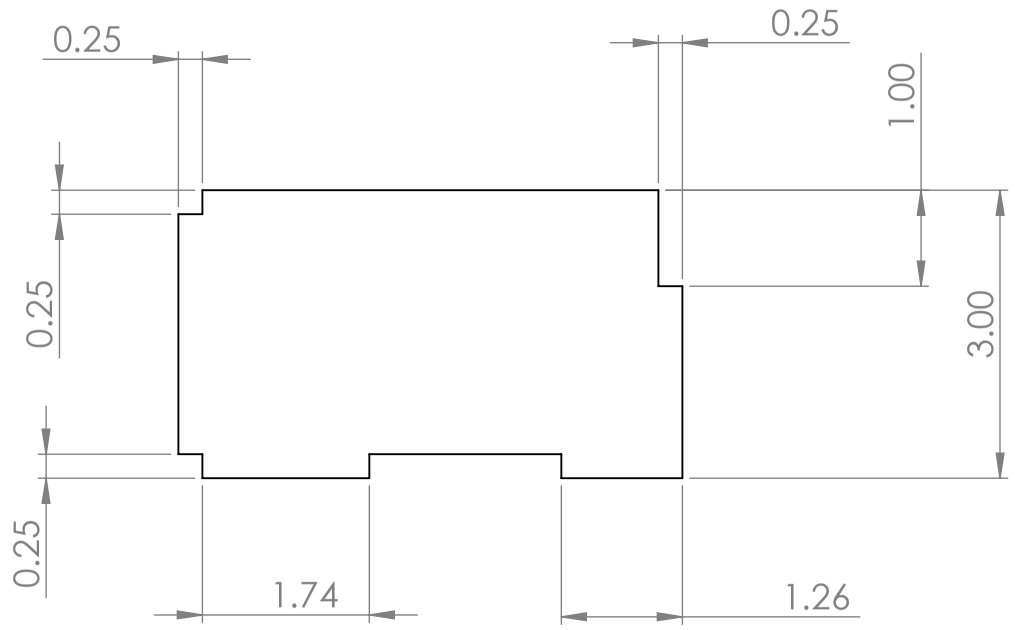

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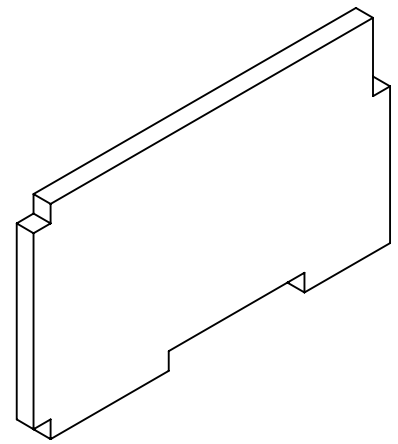

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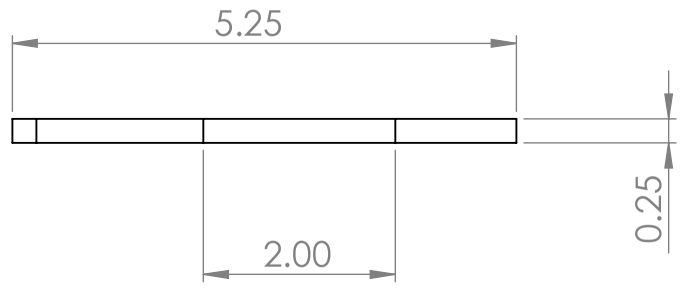

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NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

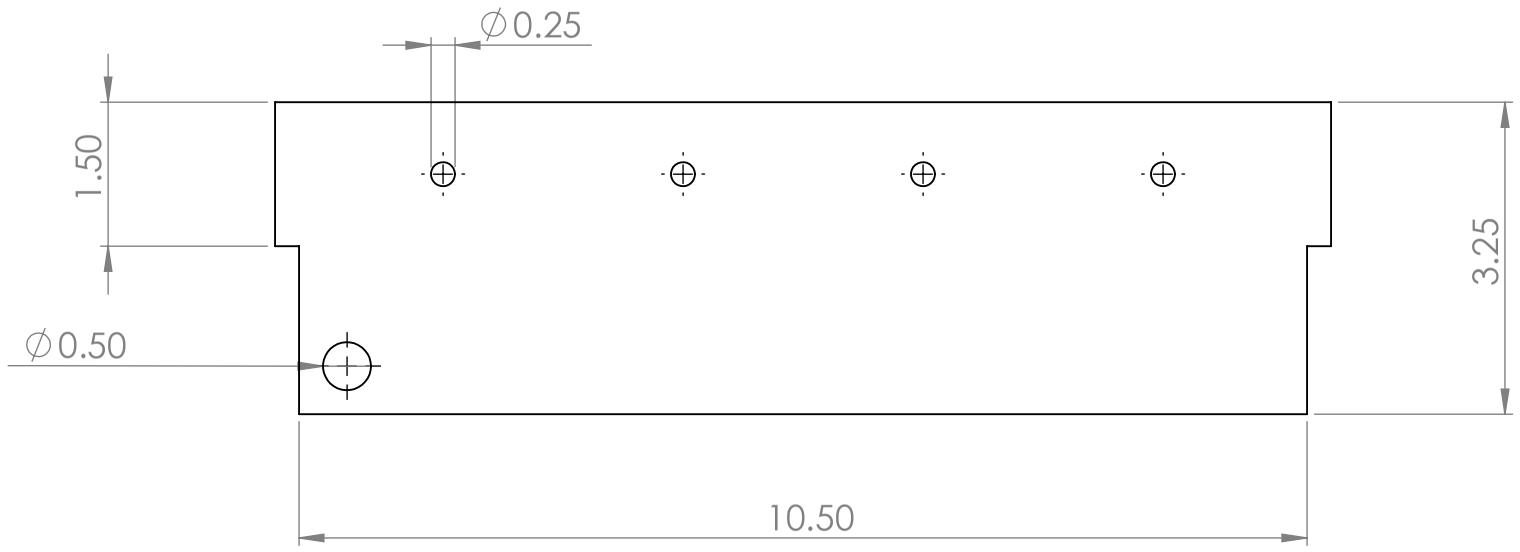
	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

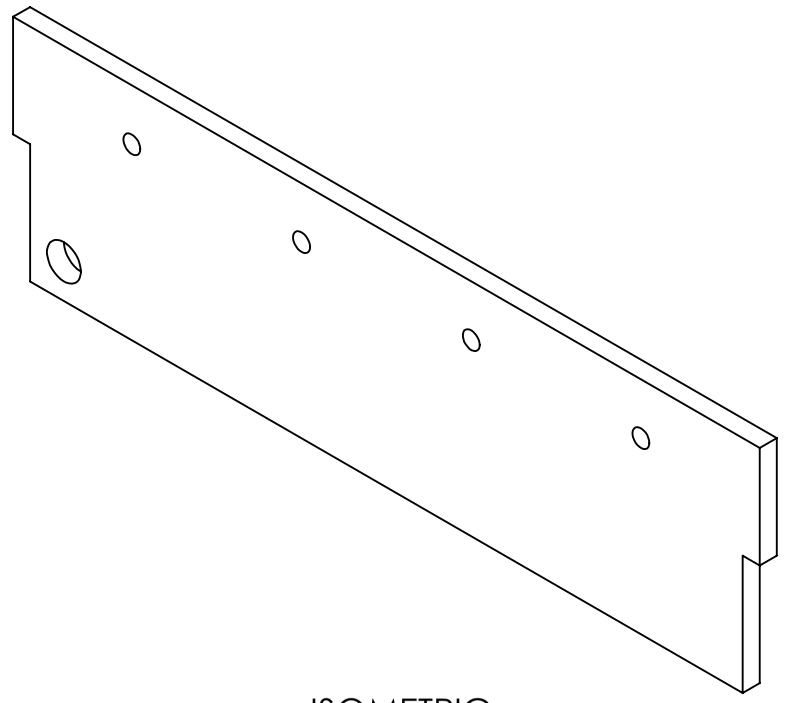
TITLE:

BOTTOM SIDE

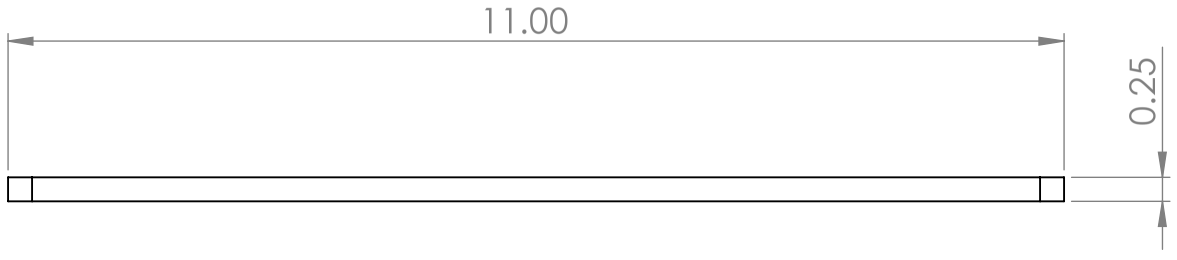
SIZE	DWG. NO.	REV
B	MQP.GRG.08	
SCALE: 1:2	WEIGHT:	SHEET 1 OF 1



FRONT



ISOMETRIC



BOTTOM

PROPRIETARY AND CONFIDENTIAL

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		UNLESS OTHERWISE SPECIFIED:
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± 0.05 ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±
		INTERPRET GEOMETRIC TOLERANCING PER:
		MATERIAL ACRYLIC
NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

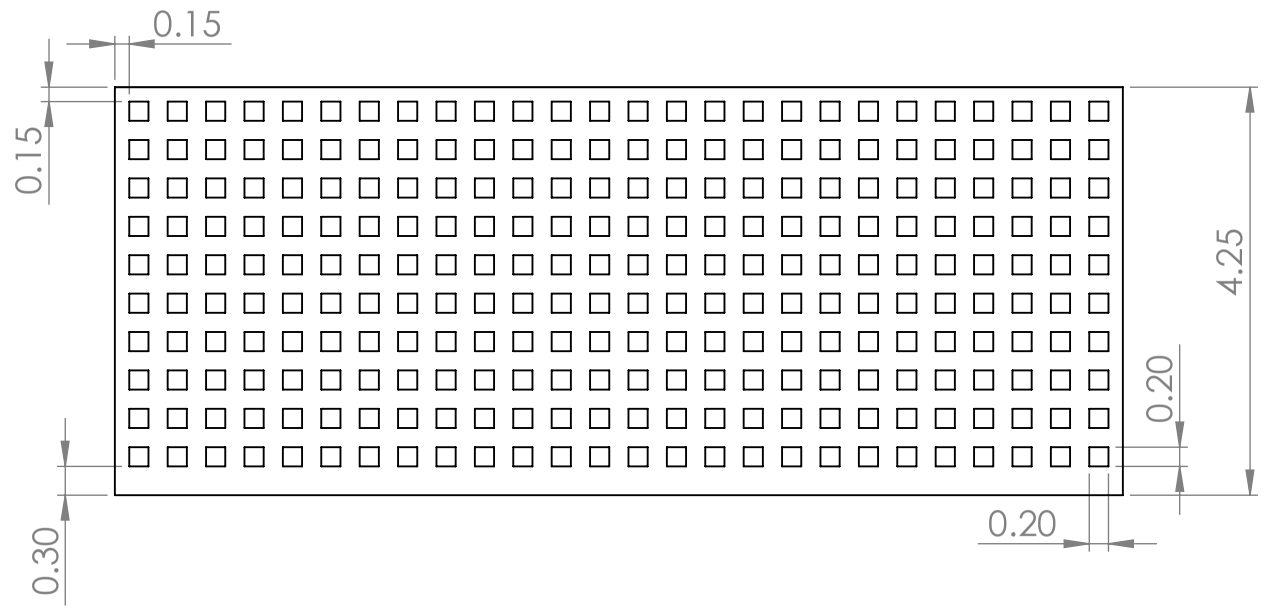
	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

TITLE:

INNER BACK

SIZE	DWG. NO.	REV
B	MQP.GRG.09	
SCALE: 1:2	WEIGHT:	SHEET 1 OF 1



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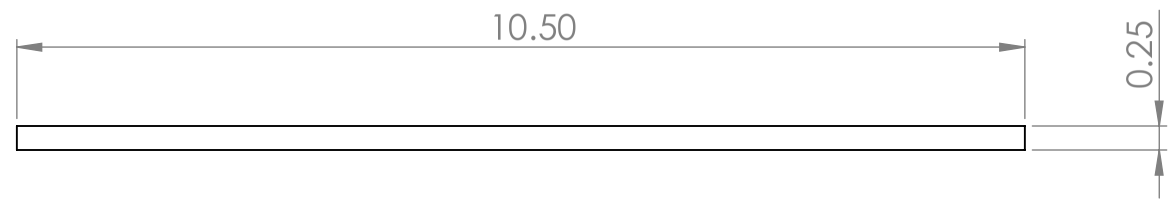

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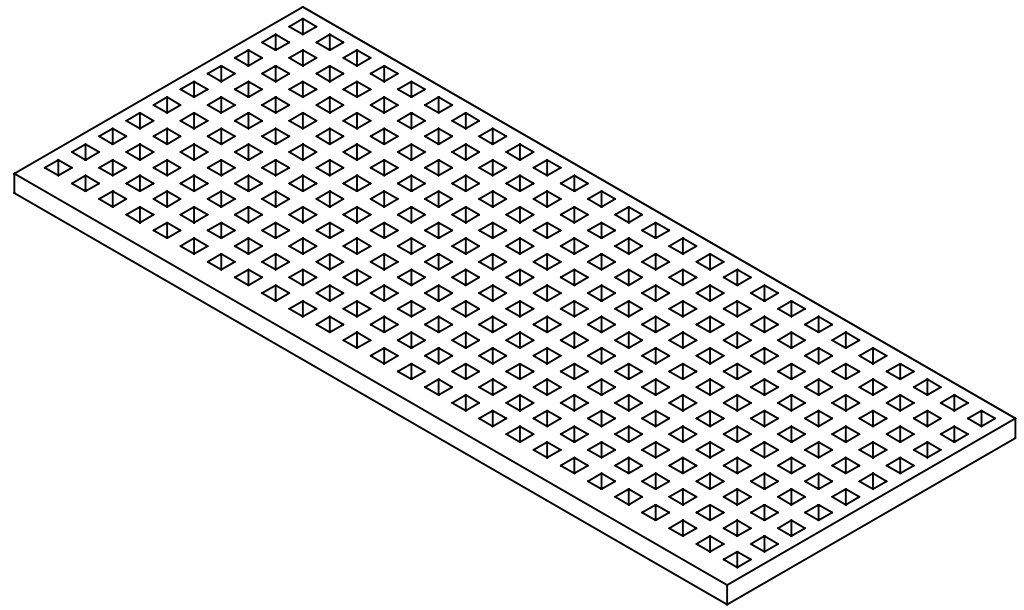


ISOMETRIC

		UNLESS OTHERWISE SPECIFIED:
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± 0.05 ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±
		INTERPRET GEOMETRIC TOLERANCING PER:
		MATERIAL ACRYLIC
NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

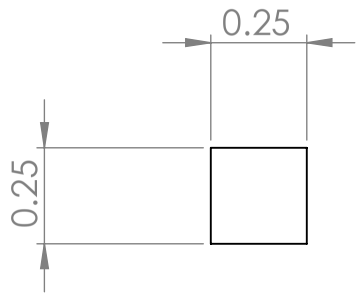
	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

TITLE:

PLATFORM

SIZE	DWG. NO.	REV
B	MQP.GRG.10	
SCALE: 1:2	WEIGHT:	SHEET 1 OF 1



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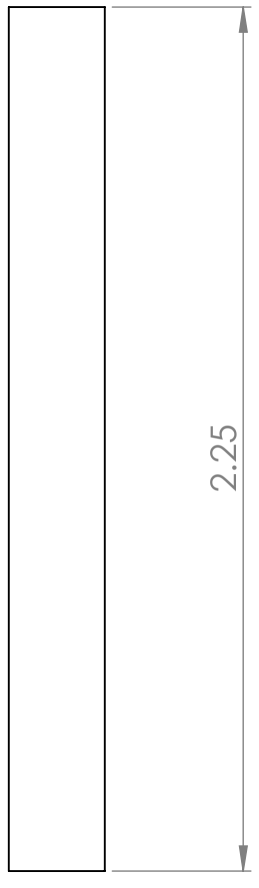

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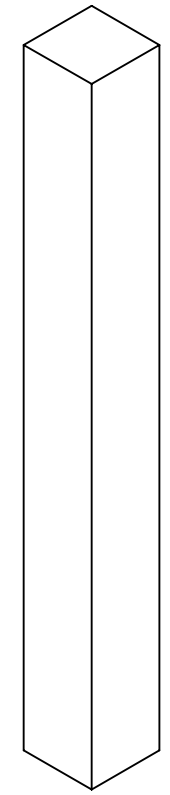


ISOMETRIC

NEXT ASSY	USED ON
APPLICATION	

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

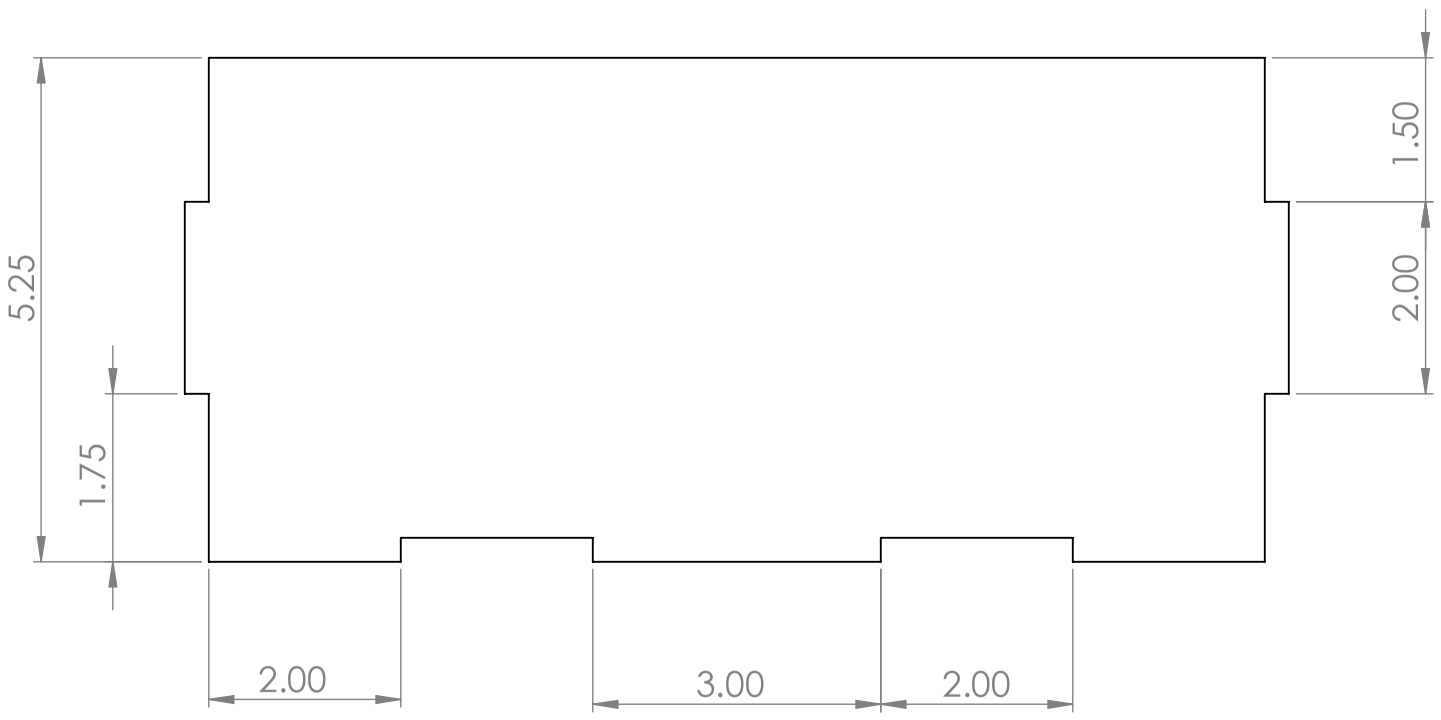
COMMENTS:

MQP ORGANS ON A VINE

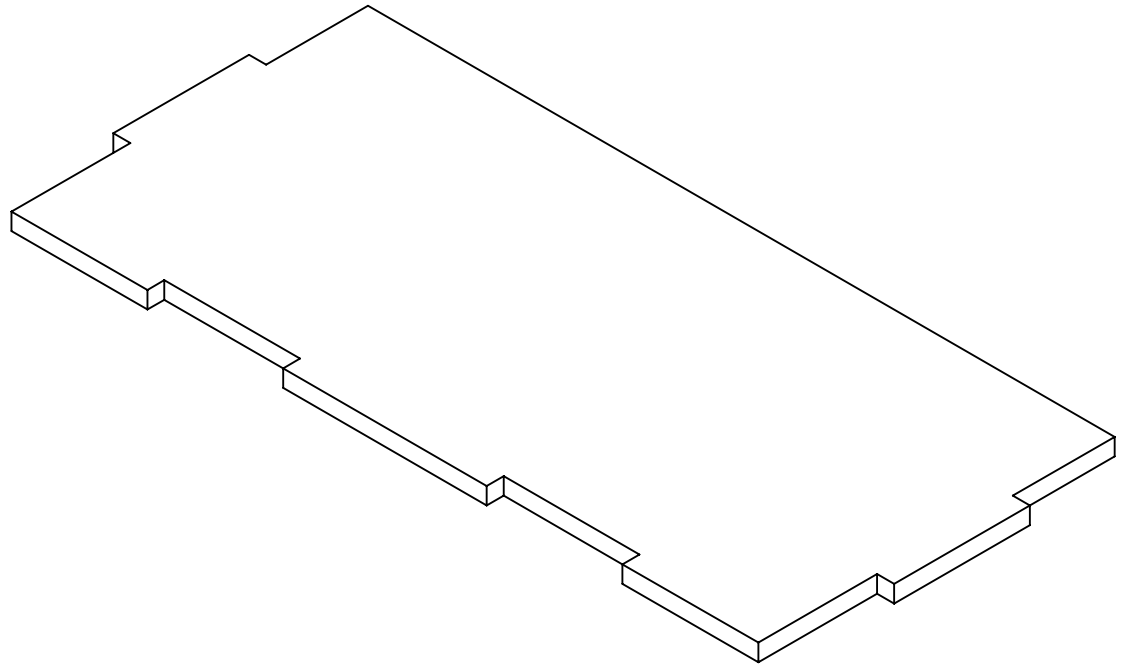
TITLE:
SUPPORT

SIZE	DWG. NO.	REV
B	MQP.GRG.11	

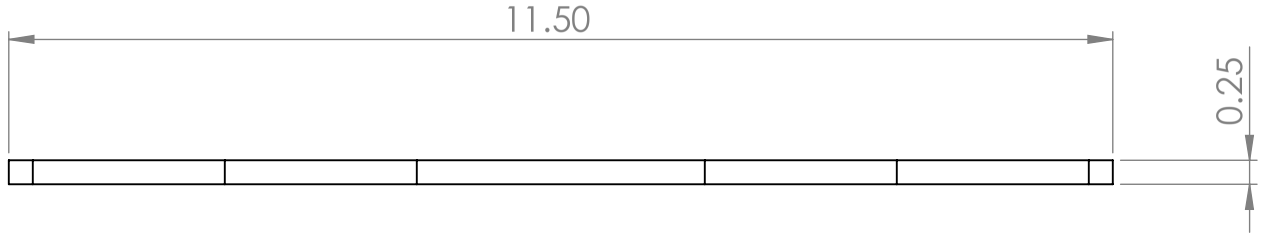
SCALE: 2:1 | WEIGHT: | SHEET 1 OF 1



TOP



ISOMETRIC



FRONT

PROPRIETARY AND CONFIDENTIAL

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INTERPRET GEOMETRIC TOLERANCING PER:	
MATERIAL	ACRYLIC
FINISH	CLEAR GLOSSY
DO NOT SCALE DRAWING	

NEXT ASSY	USED ON
APPLICATION	

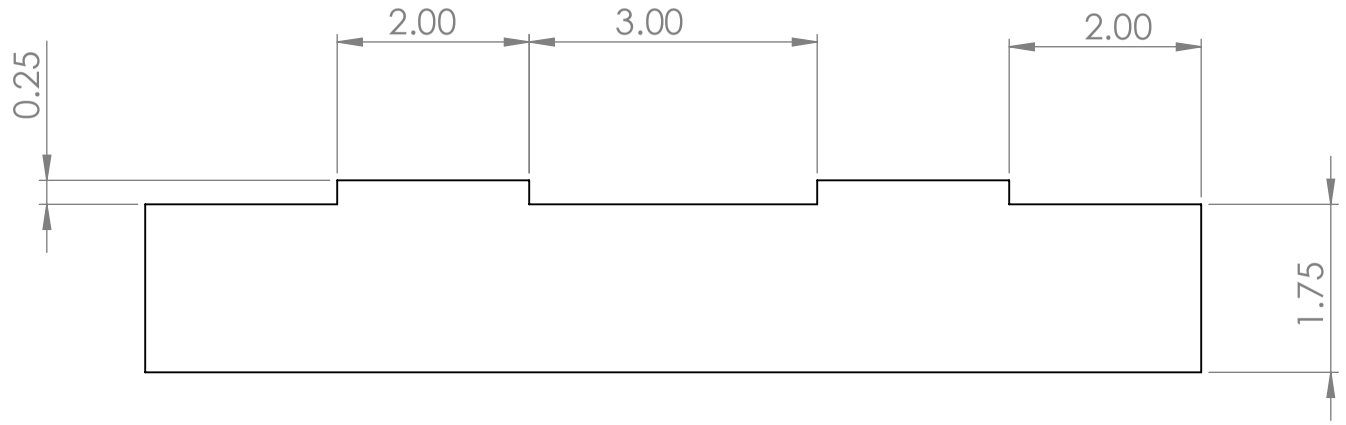
	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

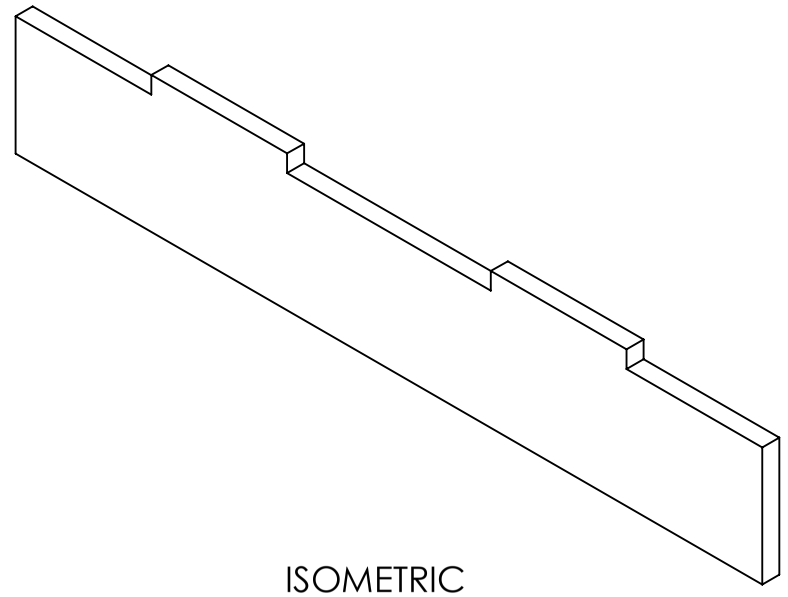
TITLE:

MAIN TOP

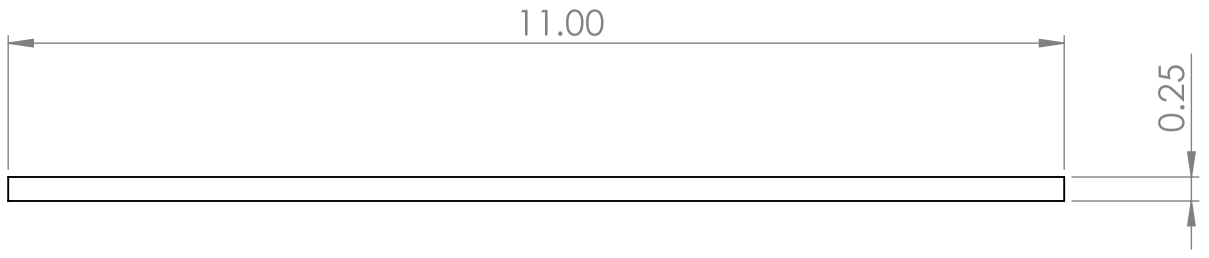
SIZE	DWG. NO.	REV
B	MQP.GRG.12	
SCALE: 1:2	WEIGHT:	SHEET 1 OF 1



FRONT



ISOMETRIC



BOTTOM

		UNLESS OTHERWISE SPECIFIED:
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± 0.05 ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±
		INTERPRET GEOMETRIC TOLERANCING PER:
		MATERIAL ACRYLIC
NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

COMMENTS:

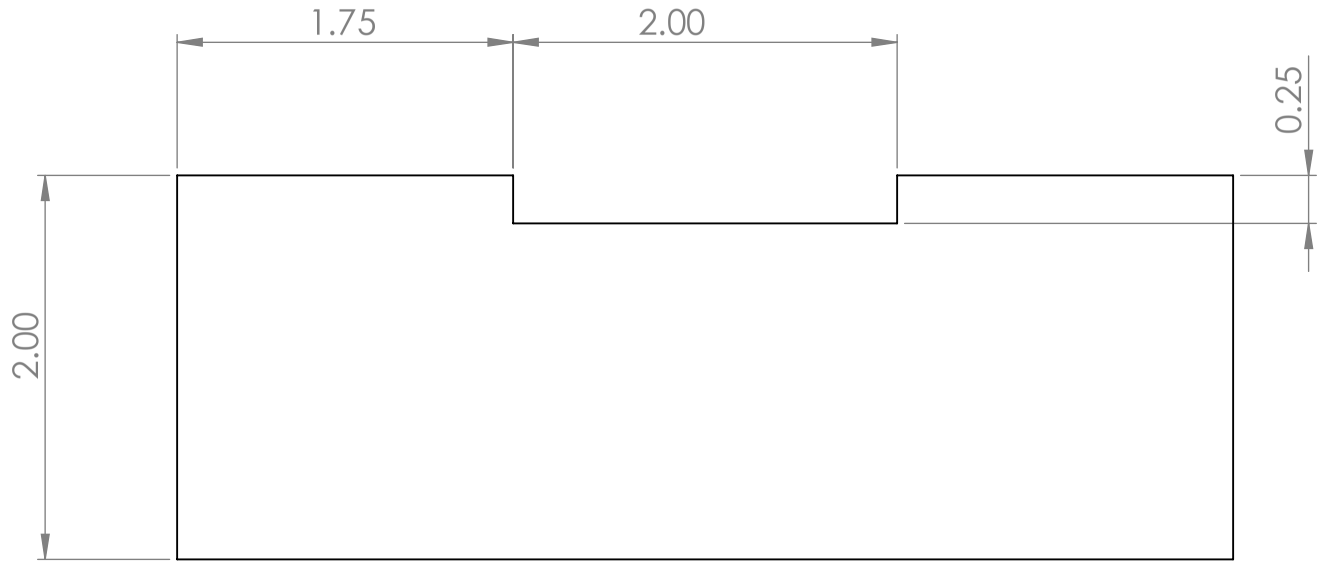
MQP ORGANS ON A VINE

TITLE:

TOP FRONT

SIZE	DWG. NO.	REV
B	MQP.GRG.13	

SCALE: 1:2	WEIGHT:	SHEET 1 OF 1



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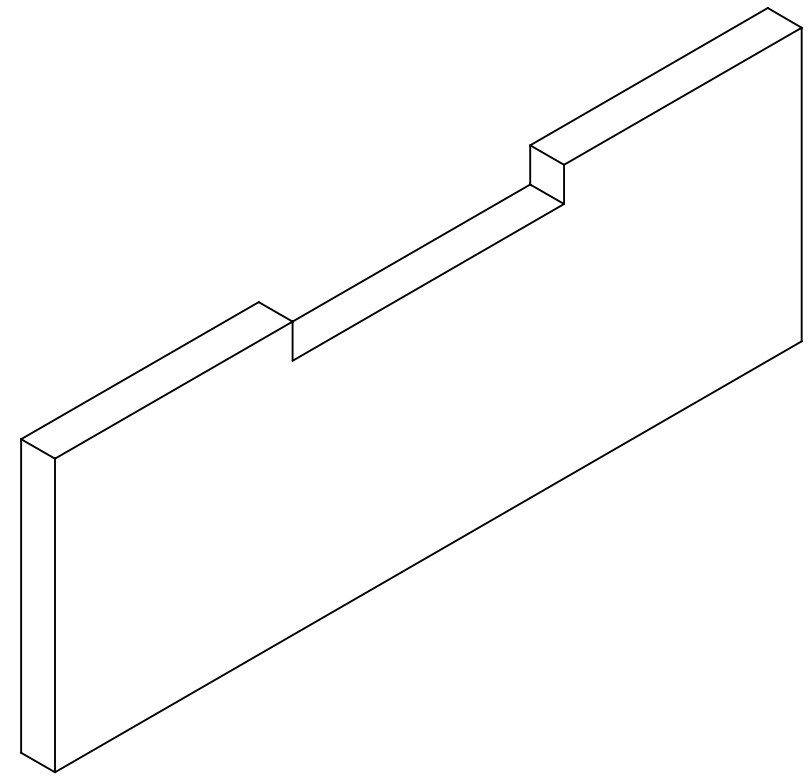
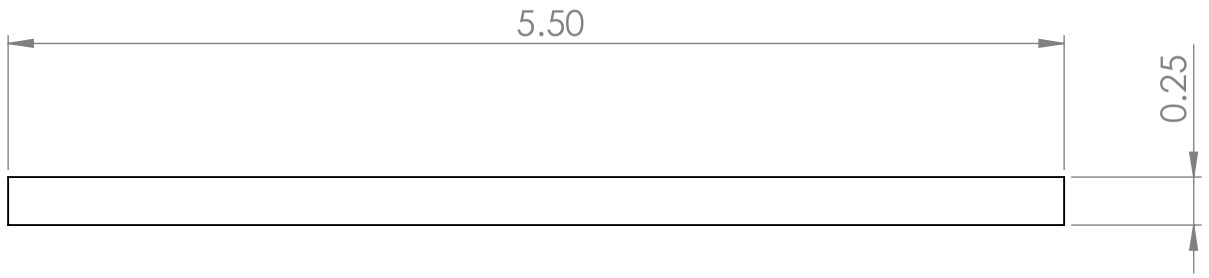


ISOMETRIC



BOTTOM

		UNLESS OTHERWISE SPECIFIED:
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± 0.05 ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±
		INTERPRET GEOMETRIC TOLERANCING PER:
		MATERIAL ACRYLIC
NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

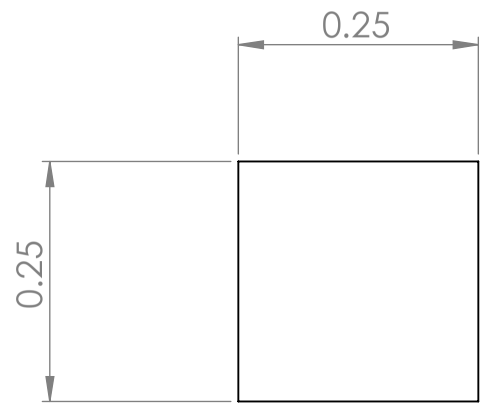
MQP ORGANS ON A VINE

TITLE:

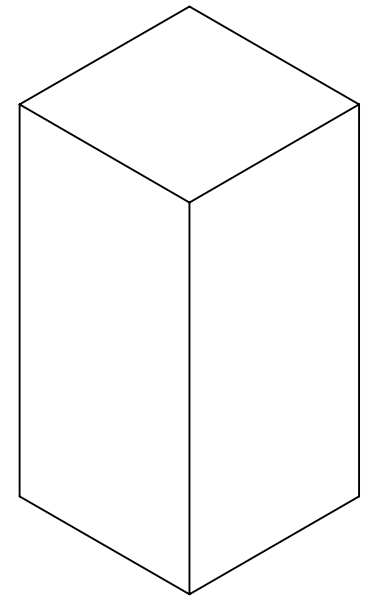
TOP SIDE

SIZE	DWG. NO.	REV
B	MQP.GRG.14	

SCALE: 1:1 | WEIGHT: | SHEET 1 OF 1



TOP



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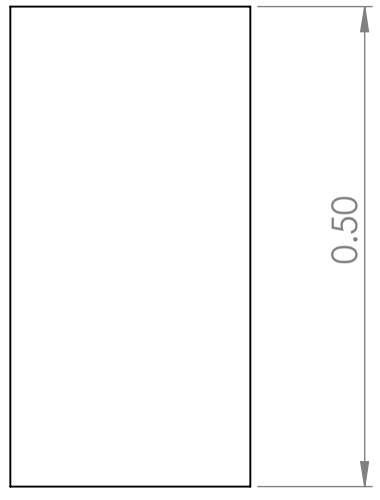


FRONT

NEXT ASSY	USED ON	
APPLICATION		DO NOT SCALE DRAWING

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

COMMENTS:

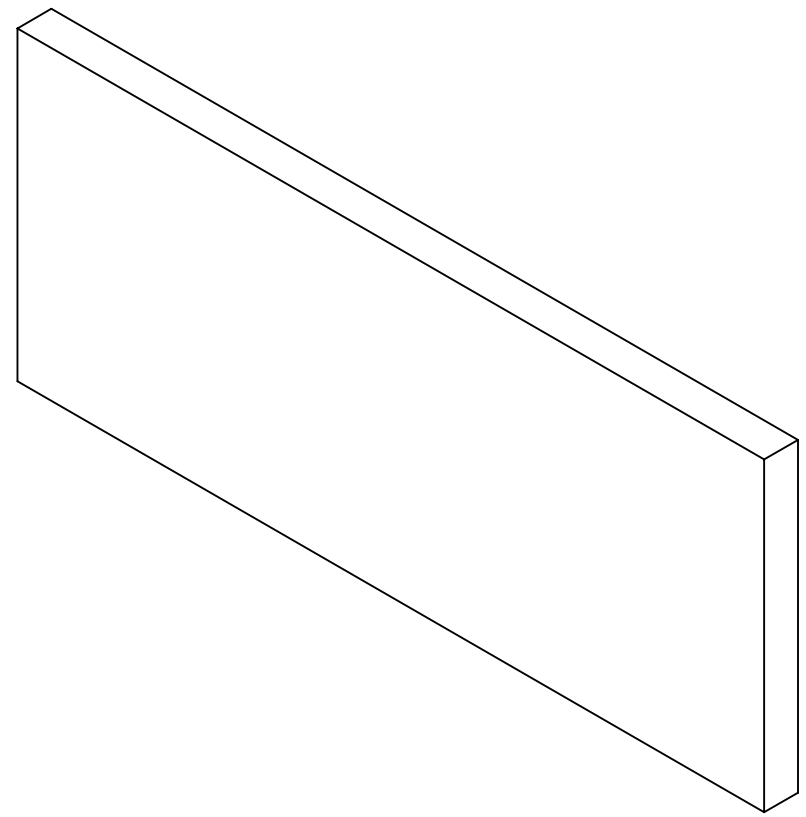
MQP ORGANS ON A VINE

TITLE:
TOP SUPPORT

SIZE	DWG. NO.	REV
B	MQP.GRG.15	

SCALE: 5:1	WEIGHT:	SHEET 1 OF 1

FRONT



ISOMETRIC

BOTTOM

		UNLESS OTHERWISE SPECIFIED:
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± 0.05 ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±
		INTERPRET GEOMETRIC TOLERANCING PER:
		MATERIAL ACRYLIC
NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

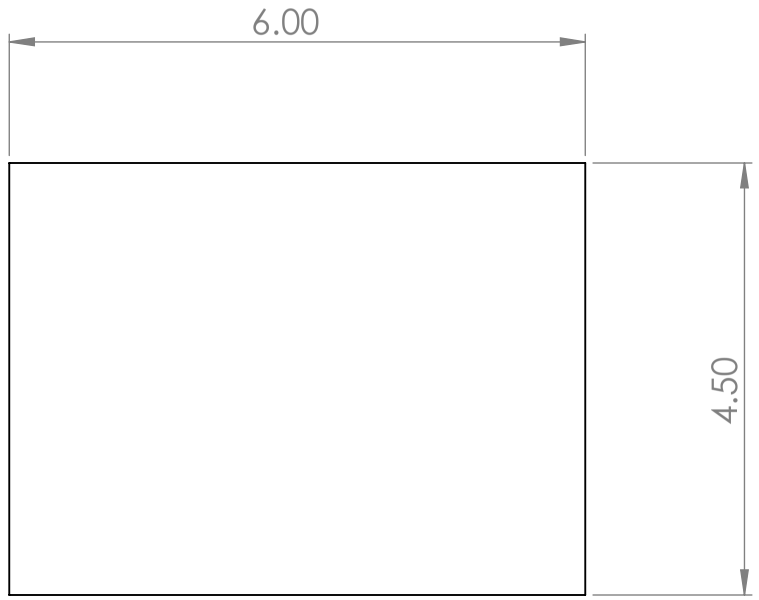
	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

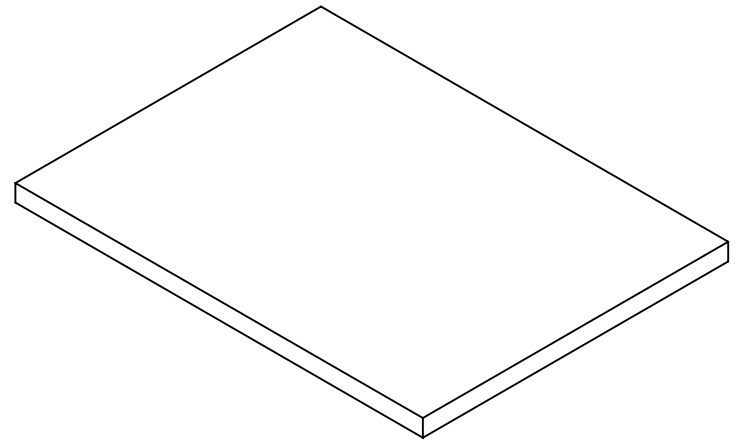
TITLE:

ELECTRIC DOOR

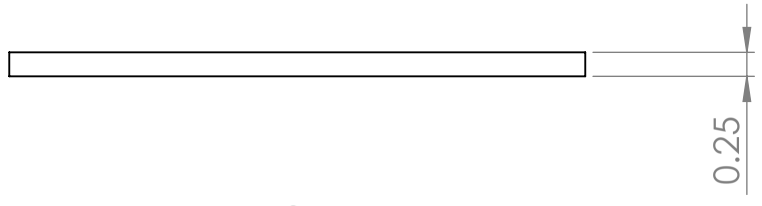
SIZE	DWG. NO.	REV
B	MQP.GRG.16	
SCALE: 1:1	WEIGHT:	SHEET 1 OF 1



TOP



ISOMETRIC



FRONT

NEXT ASSY	USED ON	
APPLICATION		DO NOT SCALE DRAWING

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

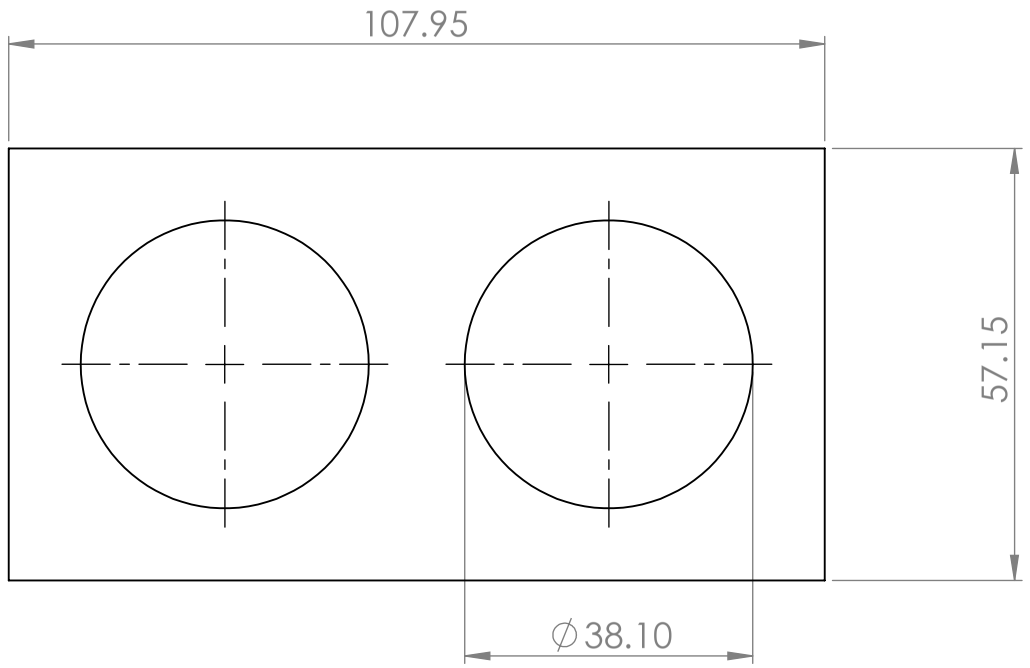
	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

COMMENTS:

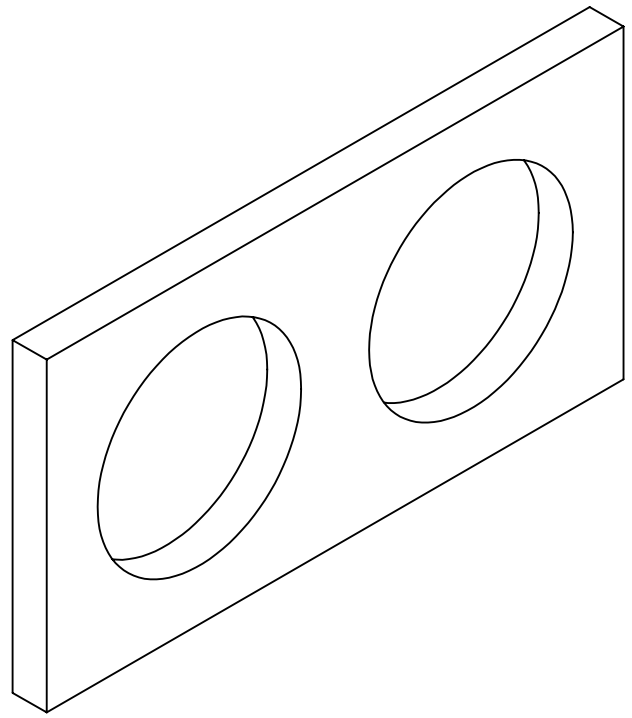
MQP ORGANS ON A VINE

TITLE:
ELECTRIC TOP

SIZE	DWG. NO.	REV
B	MQP.GRG.17	
SCALE: 1:2	WEIGHT:	SHEET 1 OF 1



RIGHT



ISOMETRIC

BOTTOM

NEXT ASSY	USED ON	
APPLICATION		

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL
ACRYLIC

FINISH
CLEAR GLOSSY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

COMMENTS:

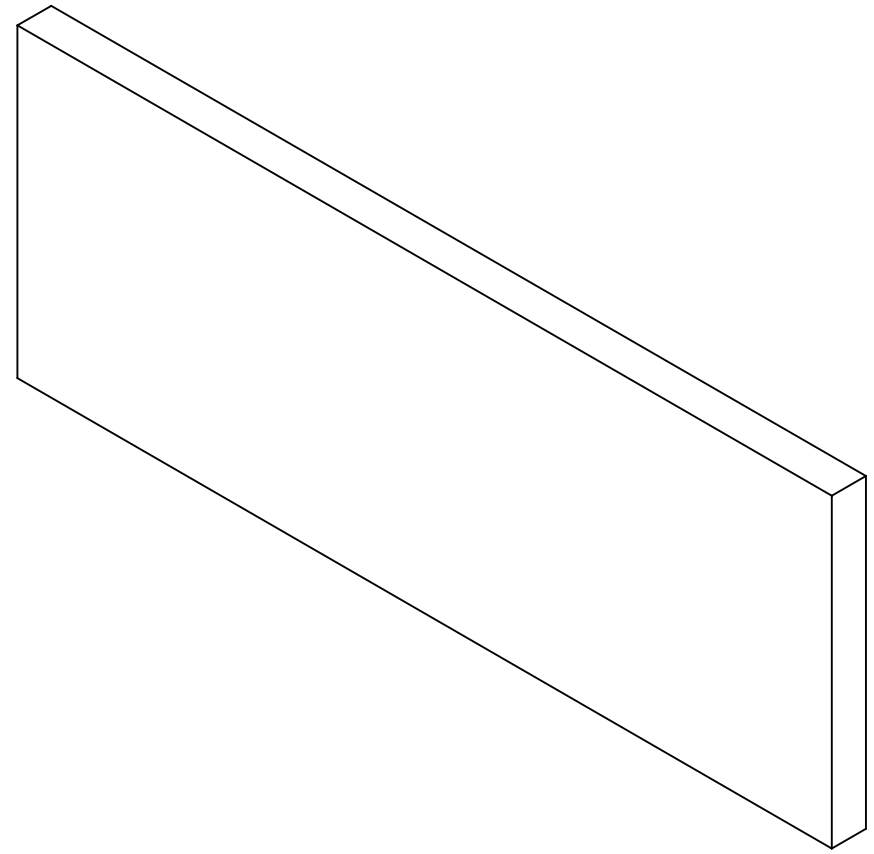
MQP ORGANS ON A VINE

TITLE:
ELECTRIC WALL

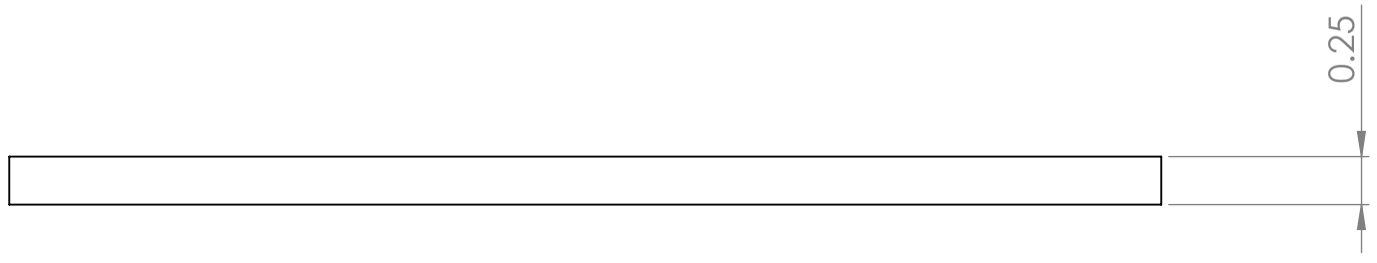
SIZE	DWG. NO.	REV
B	MQP.GRG.18	

SCALE: 1:1 | WEIGHT: | SHEET 1 OF 1

FRONT



ISOMETRIC



BOTTOM

NEXT ASSY	USED ON	
APPLICATION		

UNLESS OTHERWISE SPECIFIED:

DIMENSIONS ARE IN INCHES
TOLERANCES:
FRACTIONAL ± 0.05
ANGULAR: MACH ± BEND ±
TWO PLACE DECIMAL ±
THREE PLACE DECIMAL ±

INTERPRET GEOMETRIC TOLERANCING PER:

MATERIAL: ACRYLIC

FINISH: CLEAR GLOSSY

DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	

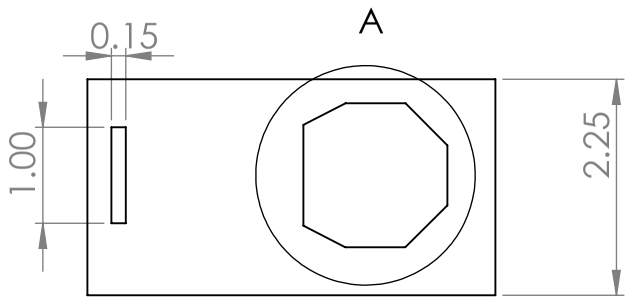
COMMENTS:

MQP ORGANS ON A VINE

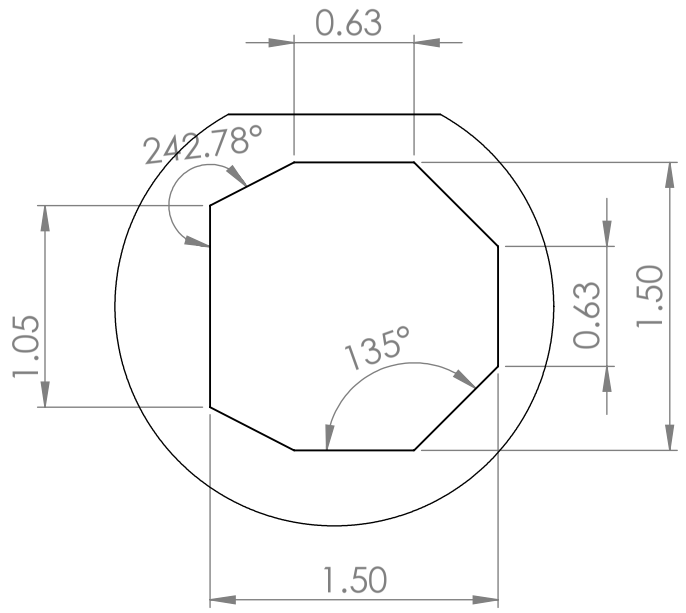
TITLE: ELECTRIC BACK

SIZE	DWG. NO.	REV
B	MQP.GRG.19	

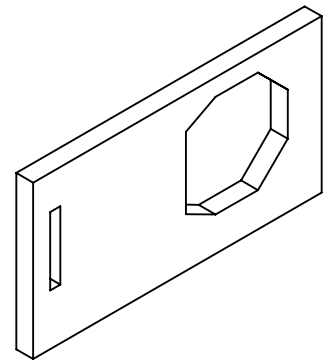
SCALE: 1:1	WEIGHT:	SHEET 1 OF 1



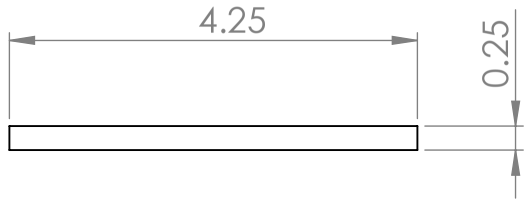
RIGHT



DETAIL A
SCALE 1 : 1



ISOMETRIC



BOTTOM

		UNLESS OTHERWISE SPECIFIED:
		DIMENSIONS ARE IN INCHES TOLERANCES: FRACTIONAL ± 0.05 ANGULAR: MACH ± BEND ± TWO PLACE DECIMAL ± THREE PLACE DECIMAL ±
		INTERPRET GEOMETRIC TOLERANCING PER:
		MATERIAL ACRYLIC
NEXT ASSY	USED ON	FINISH CLEAR GLOSSY
APPLICATION		DO NOT SCALE DRAWING

	NAME	DATE
DRAWN	JDJ	4/8/2016
CHECKED	JDJ	4/8/2014
ENG APPR.	JDJ	4/8/2014
MFG APPR.	RT	
Q.A.	LG	
COMMENTS:		

MQP ORGANS ON A VINE

TITLE:

PUMP WALL

SIZE	DWG. NO.	REV
B	MQP.GRG.20	

SCALE: 1:2	WEIGHT:	SHEET 1 OF 1